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Hepatitis C virus (HCV) genome organization.

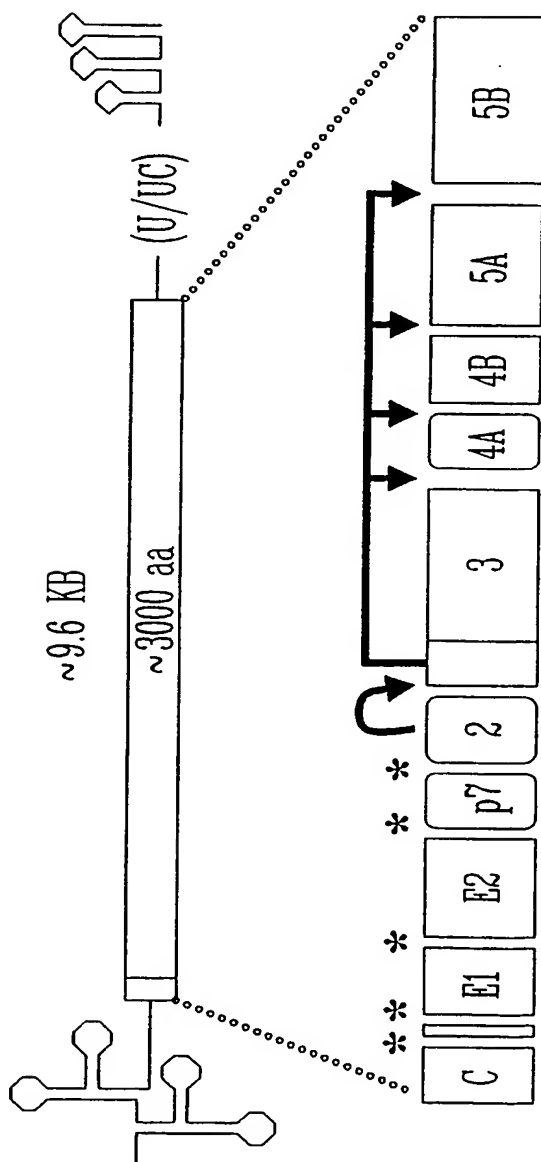


FIG. 1

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# Hypothetical model of the HCV replication cycle

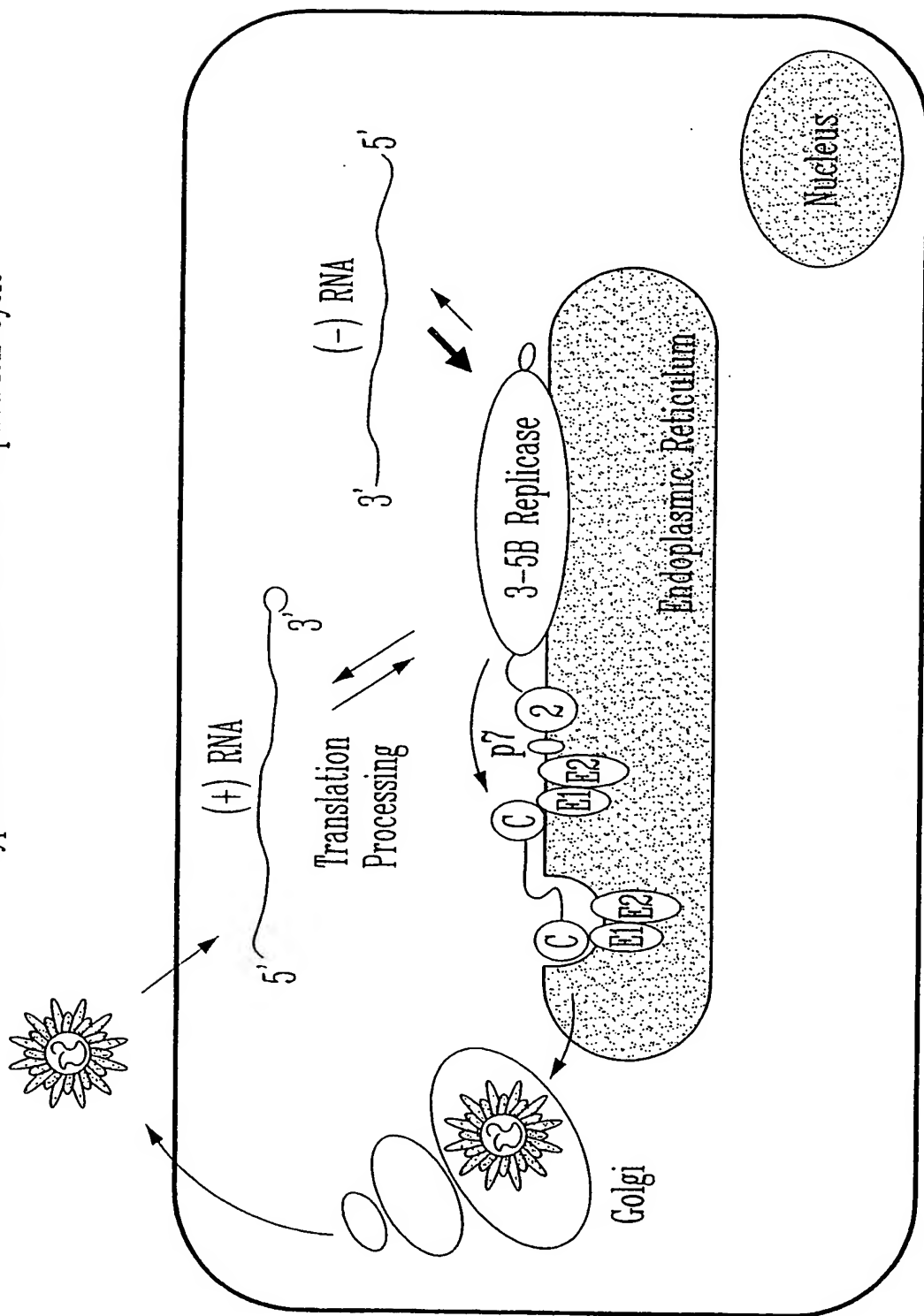


Fig. 2

**Experimental Protocol.**

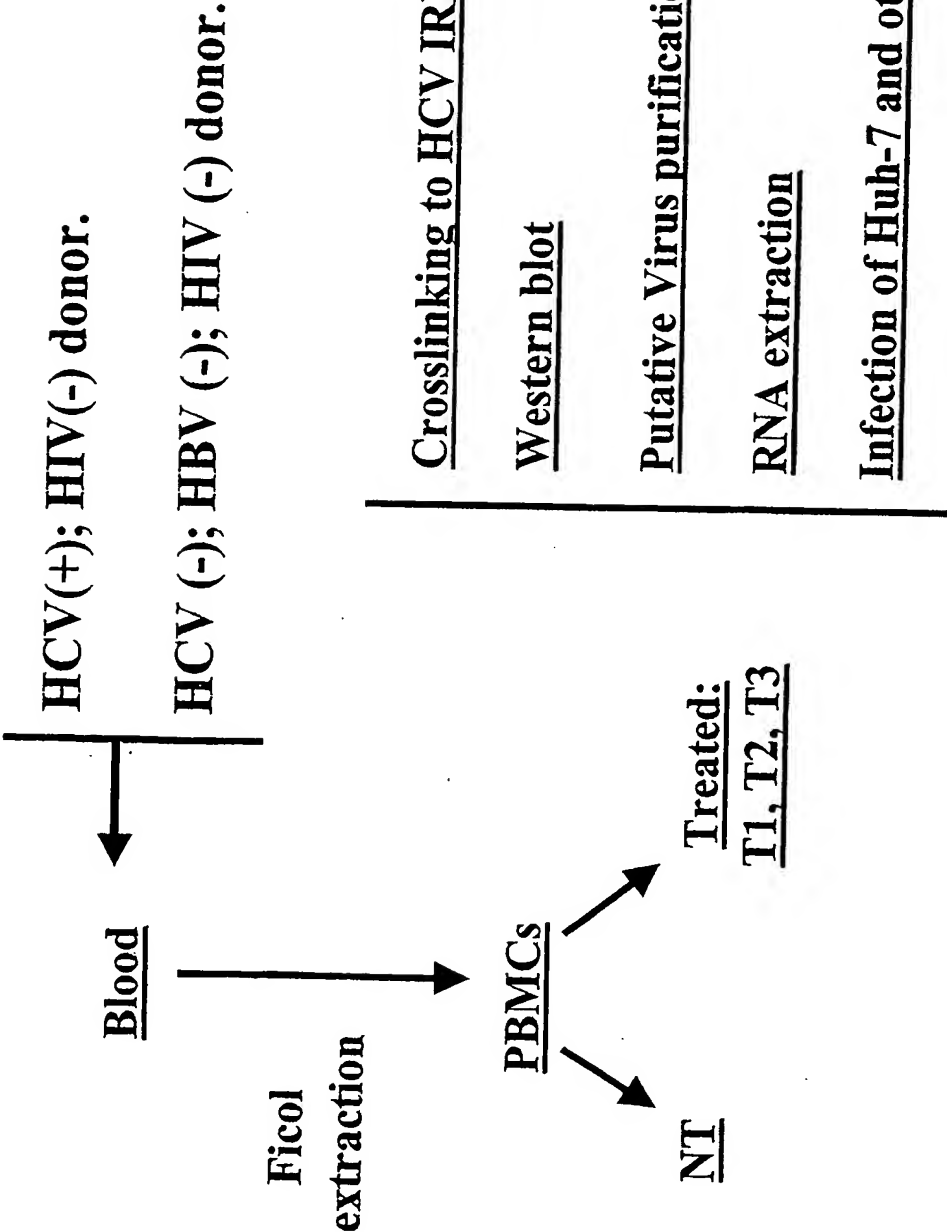
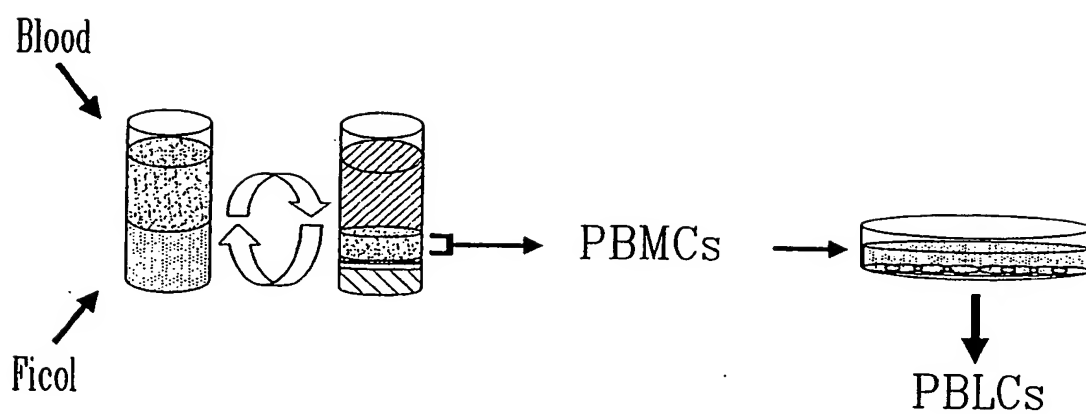


FIG. 3

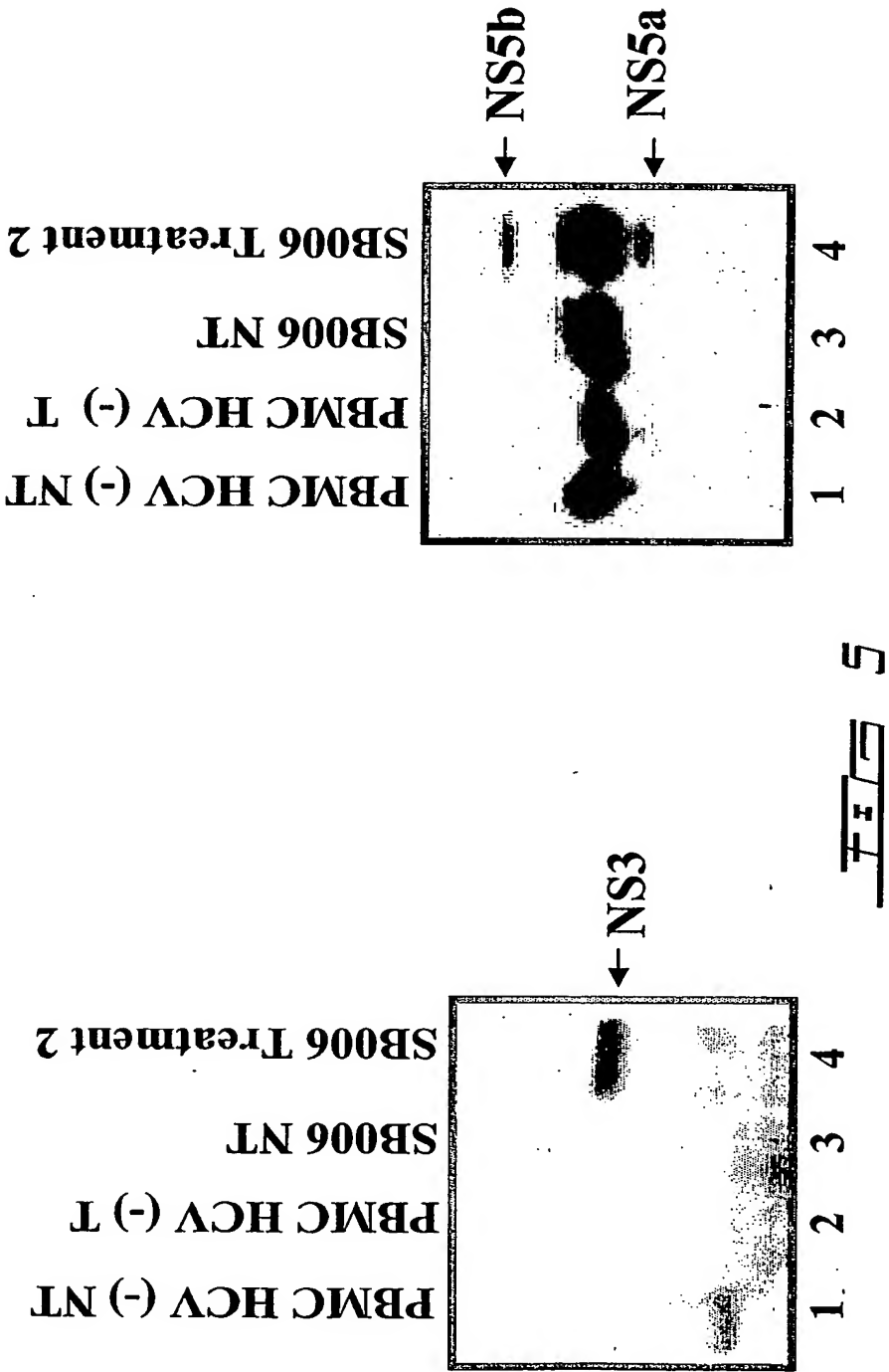
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PBMC and PBLc purification from blood samples.

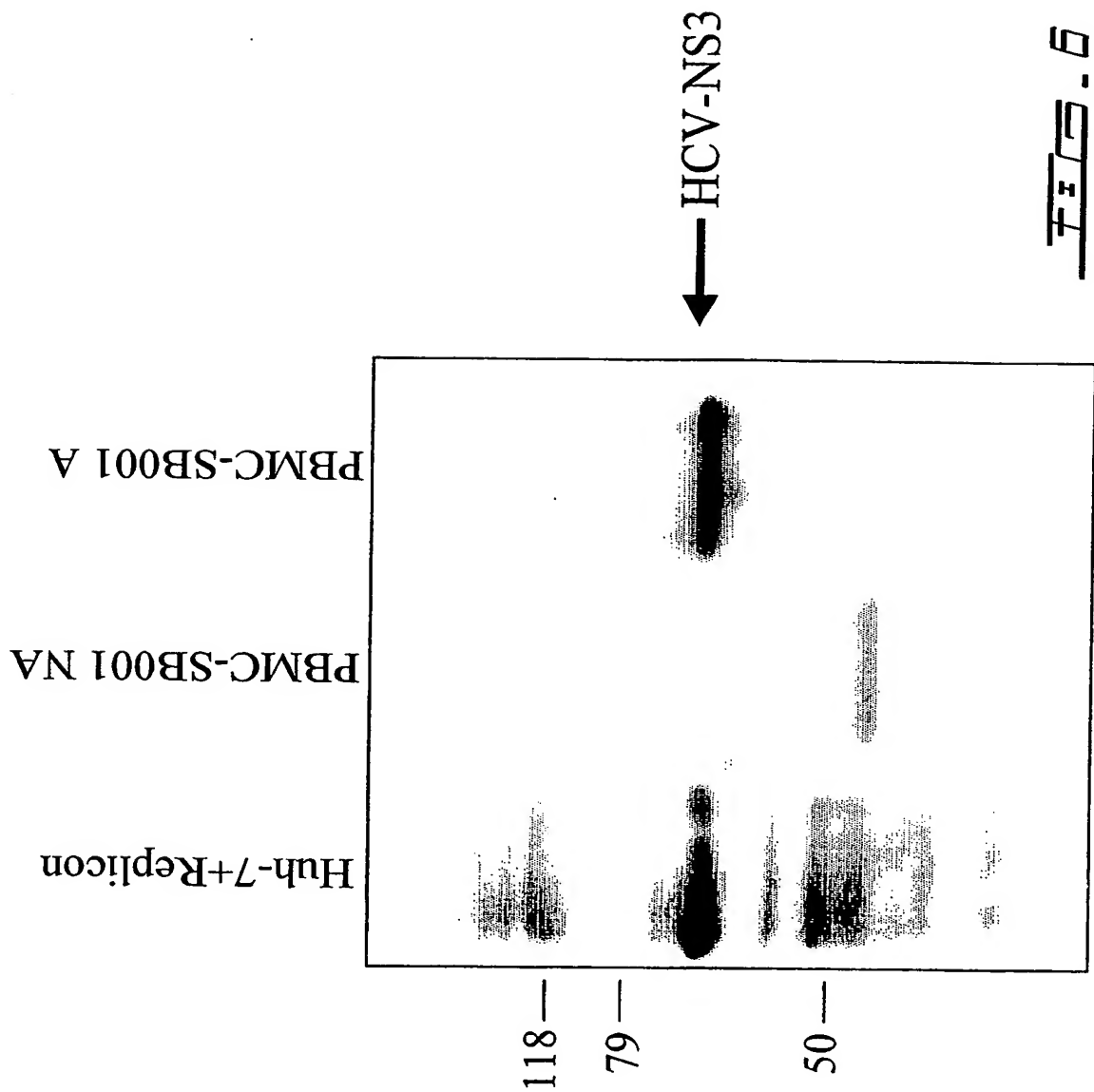
FIG. 4

Detection of HCV NS3 and NS5 proteins in cell extracts from Treated

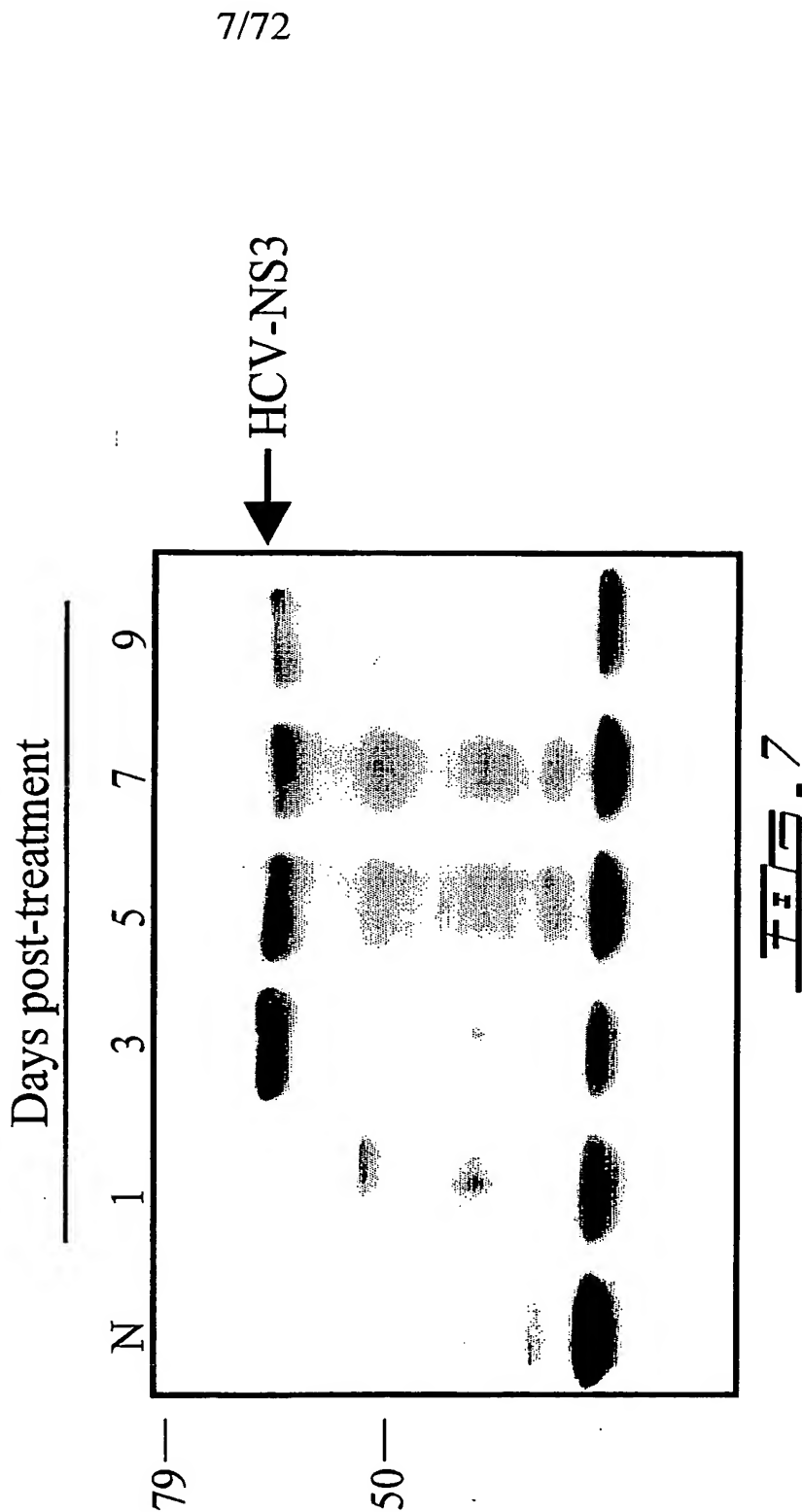
PBMC from an HCV (+) patient.  
[Boeringeranti-NS3 polyclonal antibody]



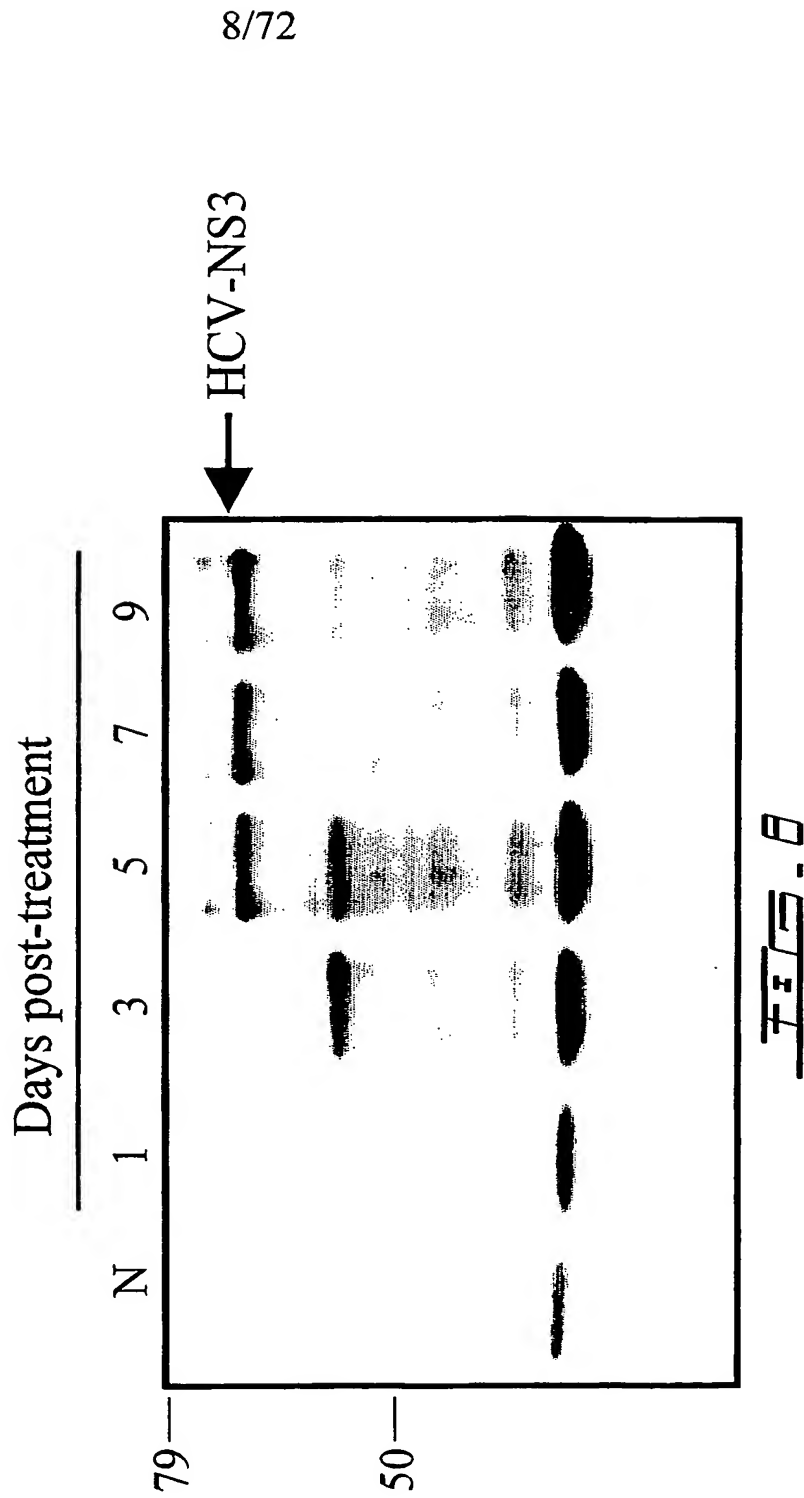
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# Time course of HCV-NS3 detection: PBMCs From patient MILL-001



# **Time course HCV-NS3 detection: PBMCs from patient MLL-002**





# Detection of HCV-NS3 protein in treated (N3) PBMCs from HCV9+) donors

PBMCs  
HCV (-) donor

Huh-7  
Huh-7+ replicon

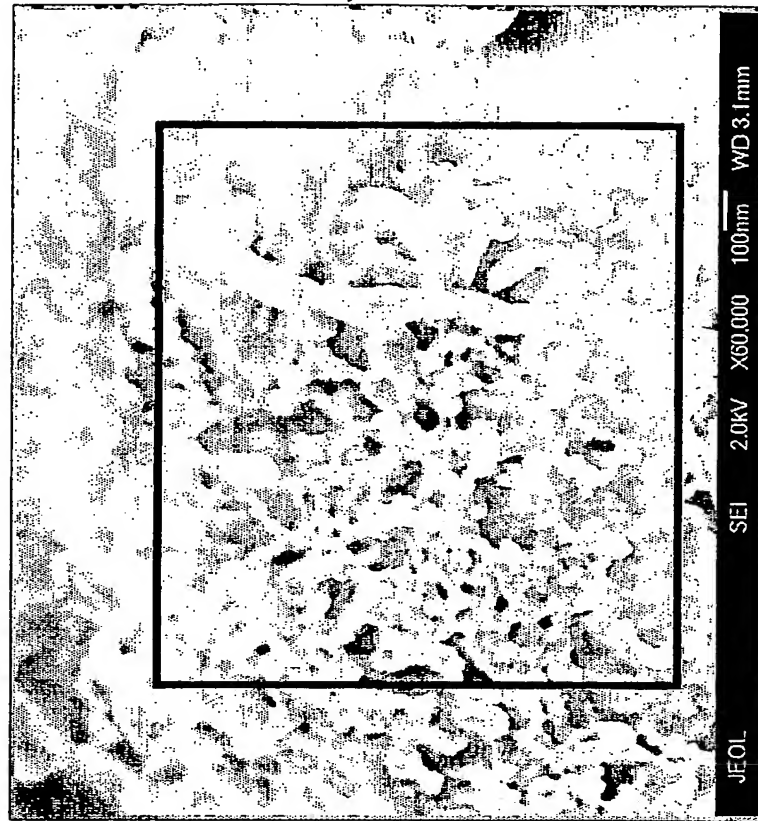
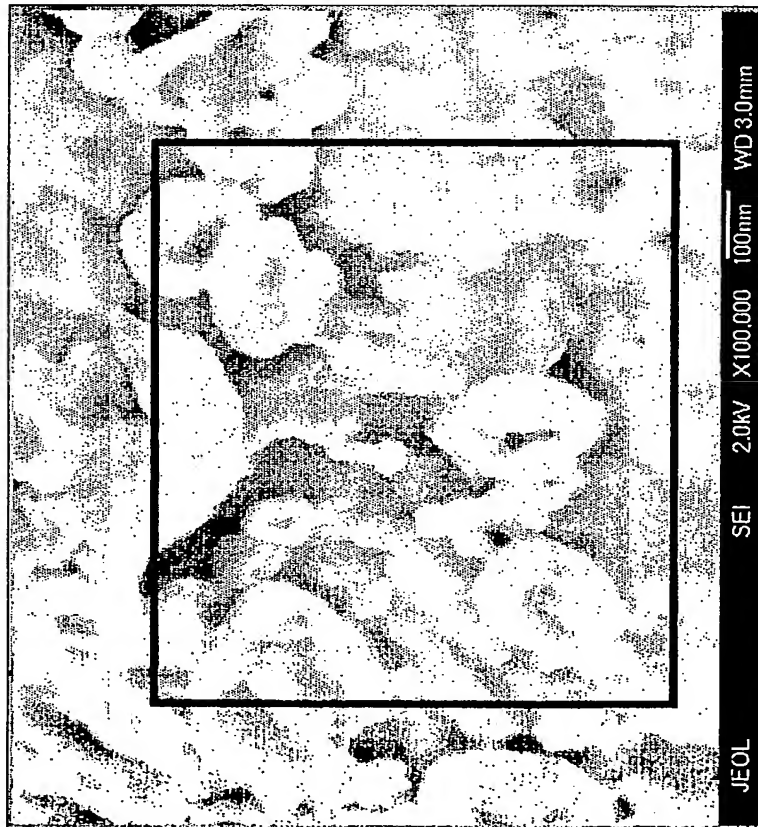
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PBMCs SB006			PBMCs SB004			PBMCs SB005		
N	A	A2	N	A1	A2	N	A1	A2

HCV-NS3

FIG. 9

Detection of virus like particles by scanning electron microscopy

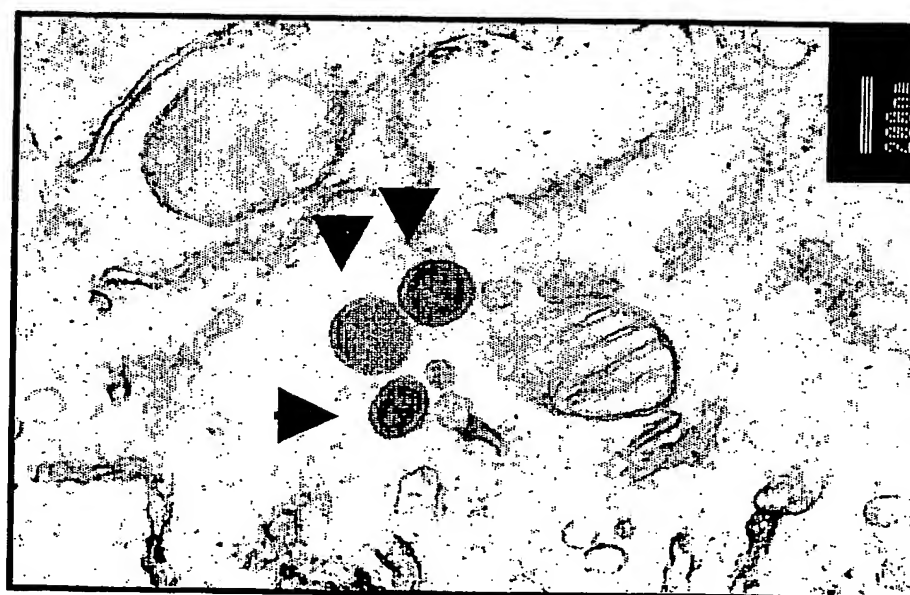


(-) Control

FEI-10

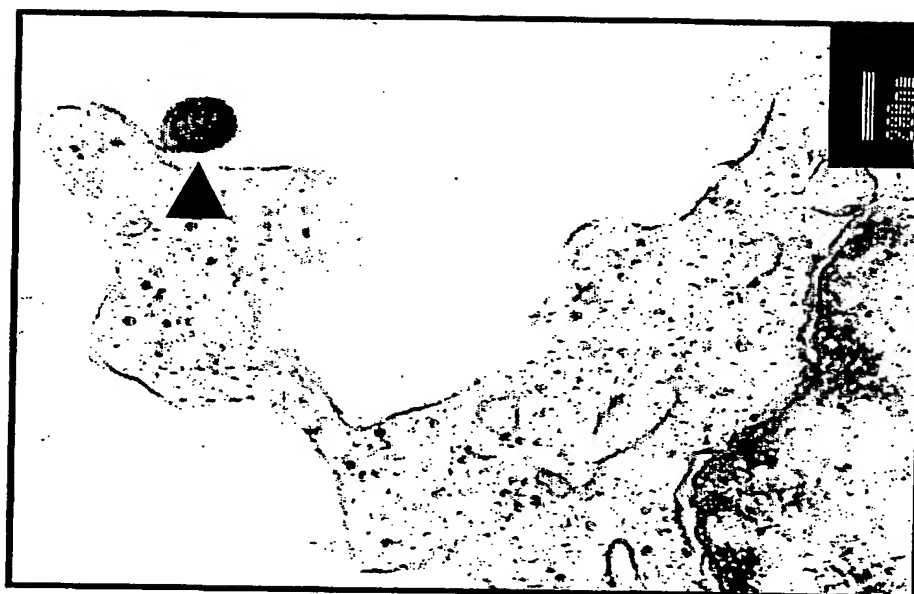
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**Electron microscopy of Activated PBLs;  
Detection of virus like particles**



200 nm

Figure 11



200 nm

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Virus partial purification.

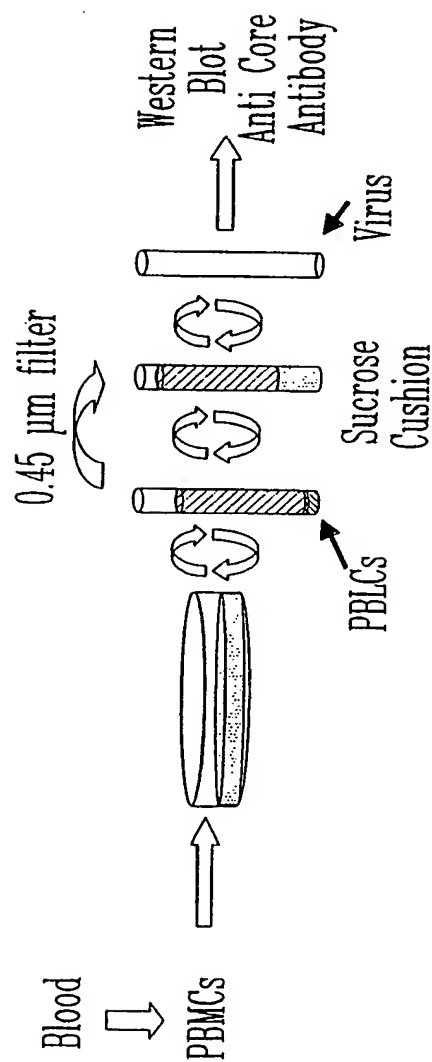
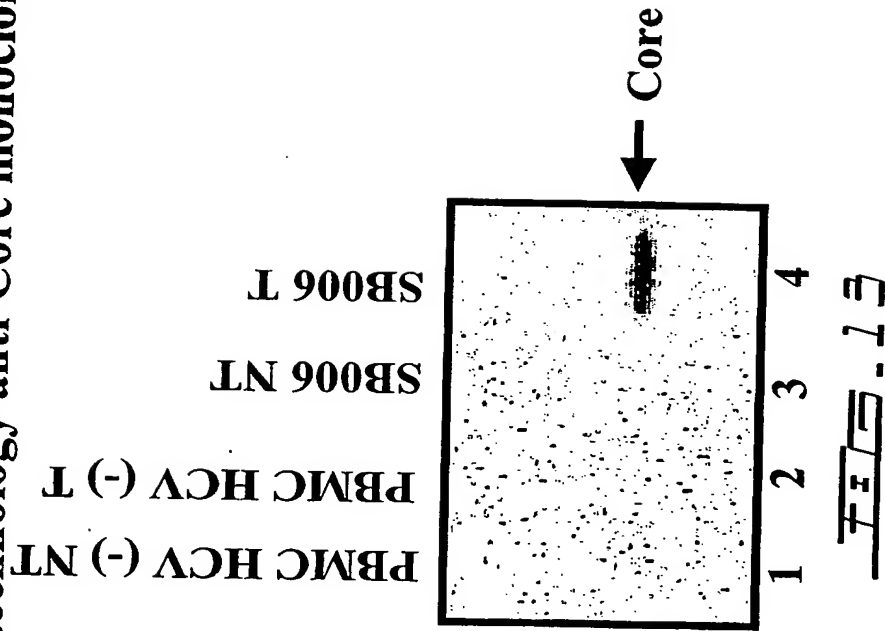


FIG. 12

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**Detection of HCV Core protein in supernatant of treated  
PBMC from an HCV (+) patient.  
[Maine biotechnology anti-Core monoclonal antibody]**



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**RNA Quantification I (virus copies/ng total RNA)**

Patient	HCV RNA In PBMC	Detection of Core (wb) in supernatant
---------	--------------------	---

After 4 days

SB004 NT

2x10<sup>3</sup>

No

SB004 T

2x10<sup>3</sup>

Yes

SB006 NT

1.8 x10<sup>3</sup>

No

SB006 T

2x10<sup>2</sup>

Yes

After 20 days

SB004

0.00

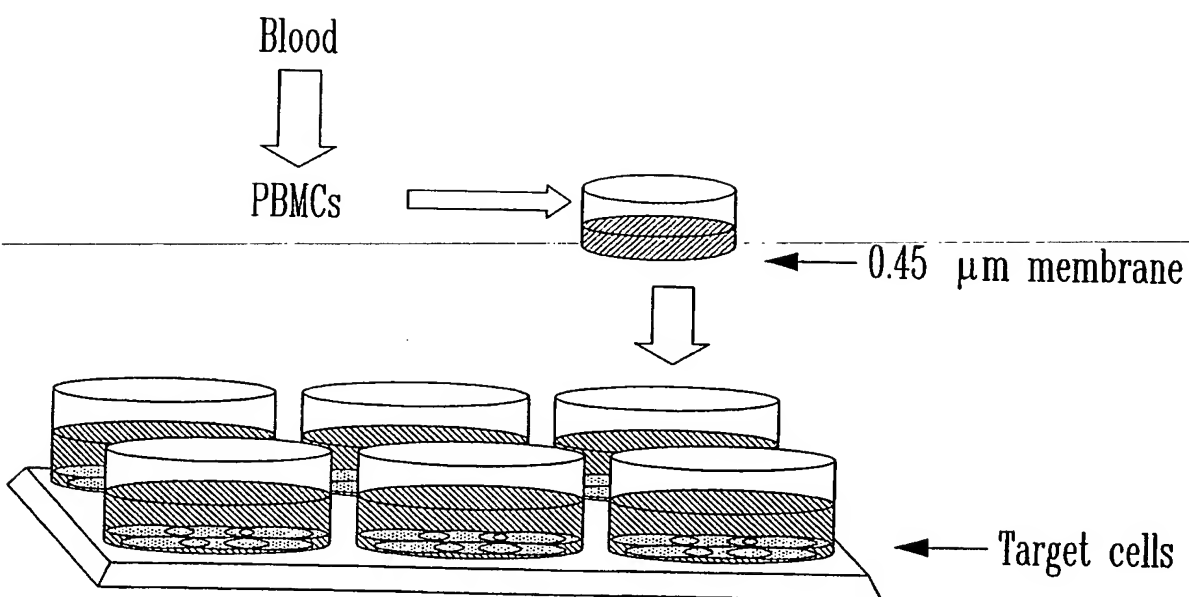
SB006

0.00

FEF - 14

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## Infection assay; co-culture

FIG. 15

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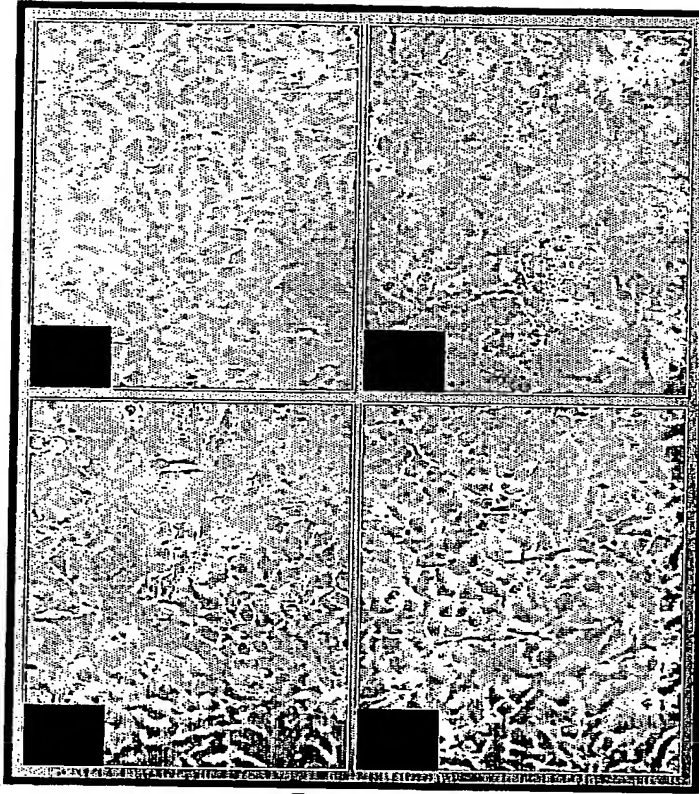
Infection of MT-4 cells RNA Quantification II (virus copies/ng total RNA)			
Patient	HCV RNA In PBMC	Detection of Core (wb) in supernatant	HCV RNA In MT-4
<u>After 10 days</u>			
SB001 NT	13	No	0.00
SB001 T	12	Yes	1600
<u>After 20 days</u>			
SB001	0.00		0.00
SB001	0.00		0.00

~~TABLE - 16~~



## Co-culture of Huh-7 and HCV (-) PBMCs.

- 1- Huh-7
- 2- Huh-7 + PBMCs HCV (-) NT
- 3- Huh-7 + Treatment
- 4- Huh-7 + PBMCs HCV (-) T



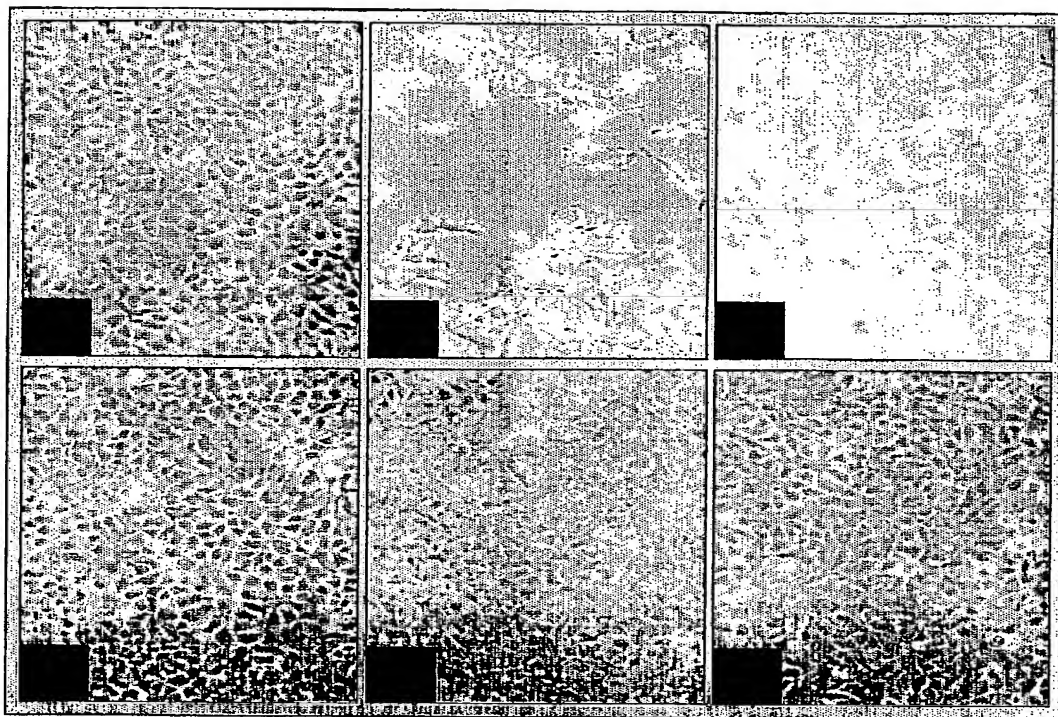
FILE - 17

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# Co-culture of Huh-7 and HCV (+) PBMS<sup>o</sup> Cs (SB0006).

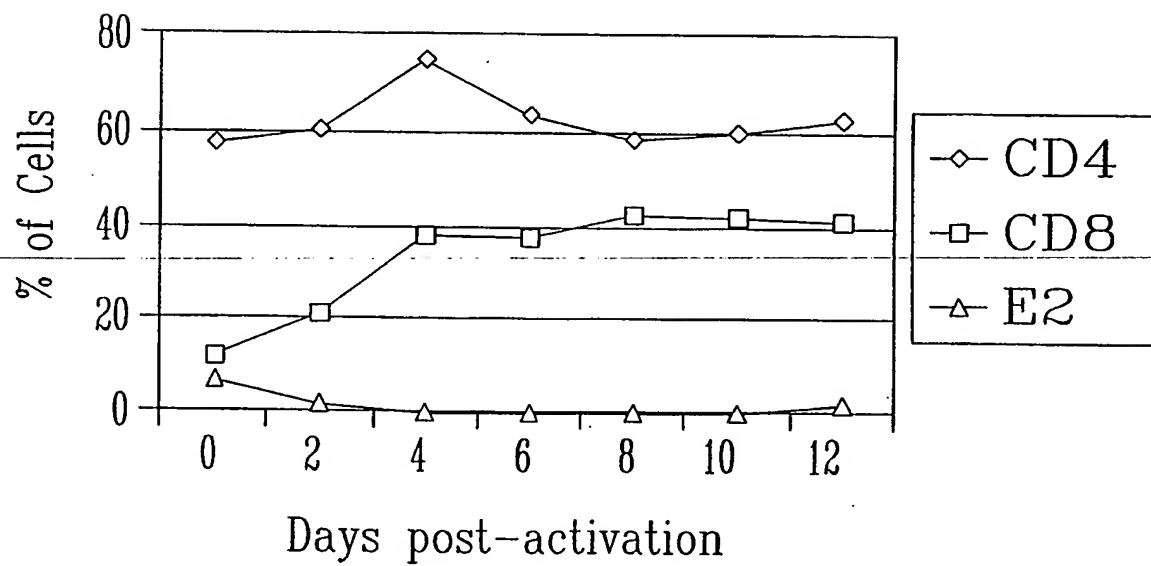
1. Huh-7
- 2-3. Huh-7 + PBMCs HCV (+) NT
4. Huh-7 + Treatment
- 5-6. Huh-7 + PBMCs HCV (+) T

FIG. 18

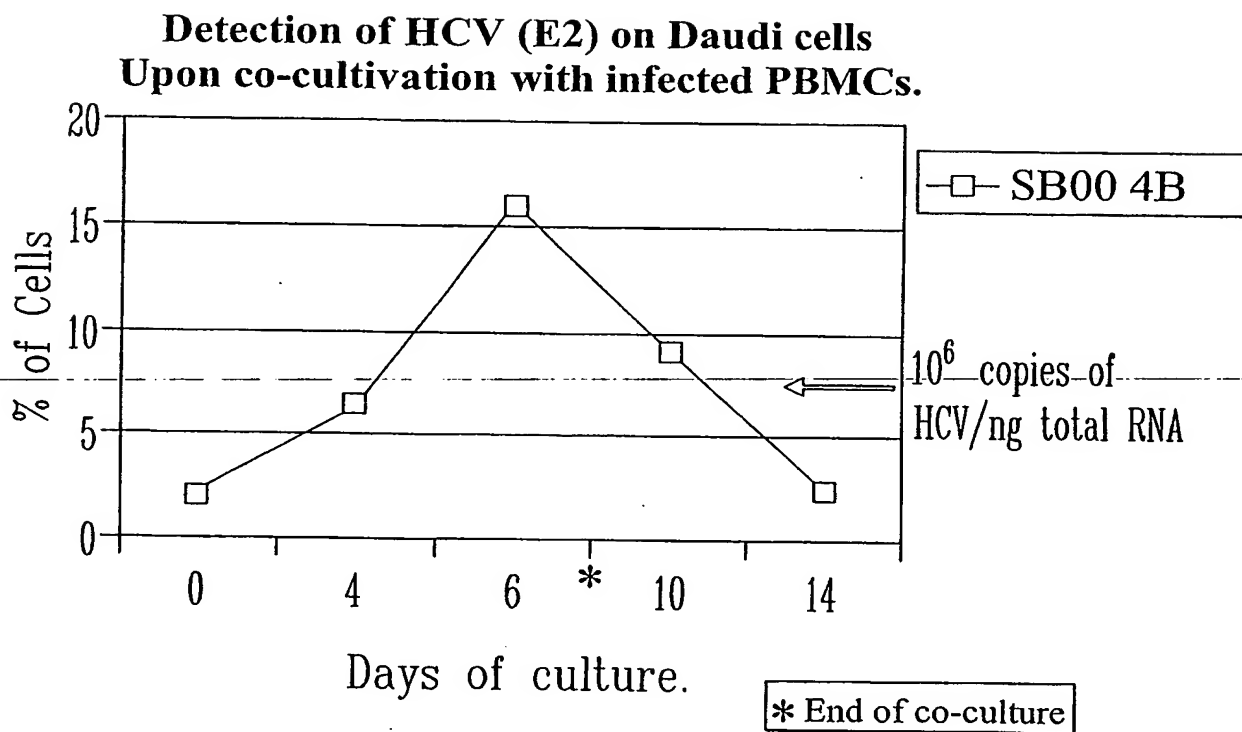


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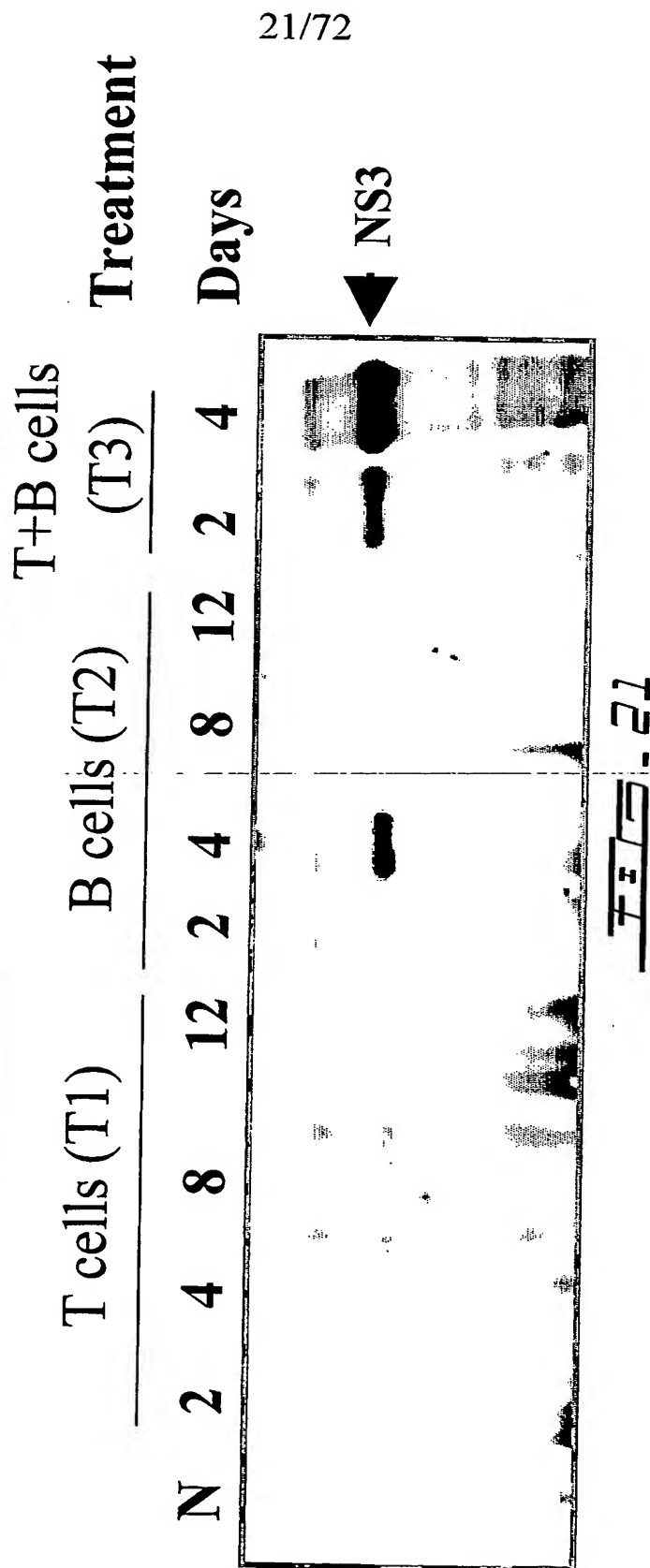
PHA Activation of PBMCs from patient SB004;  
HCV is not in T cells

FIG. 19

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FIG. 20

**Comparison of different activation treatments;**  
**PBMCs from donor MLL-010**



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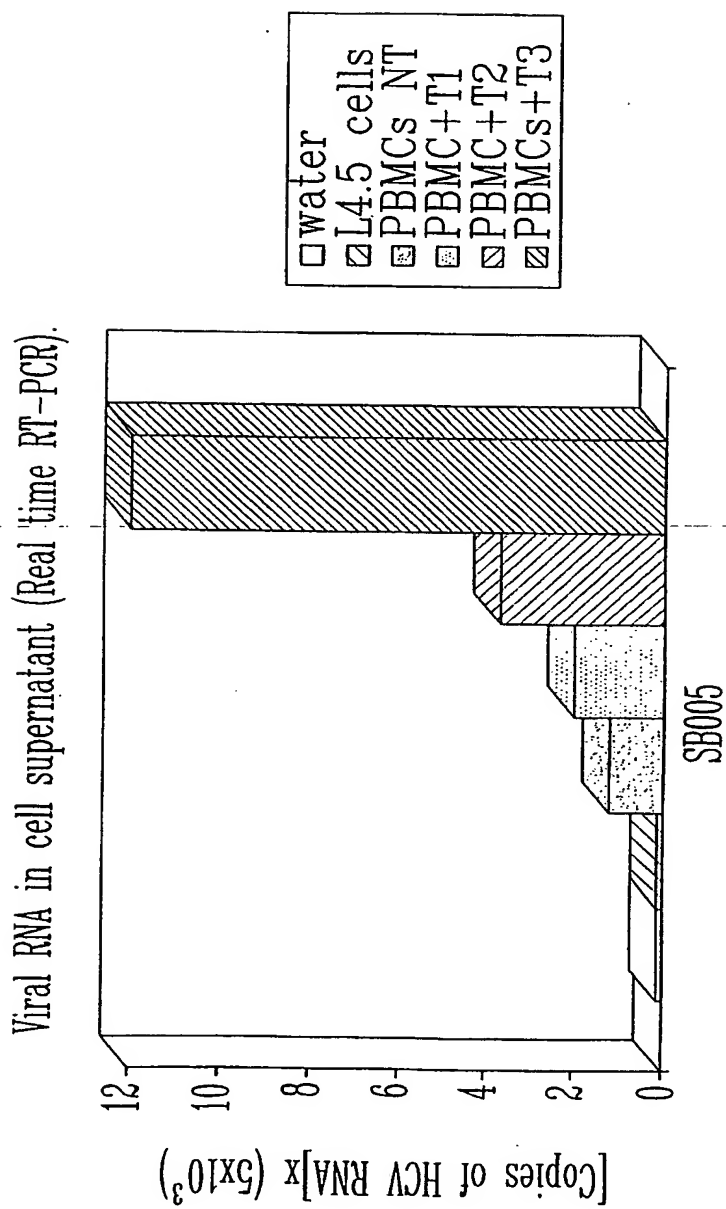


FIG. 22

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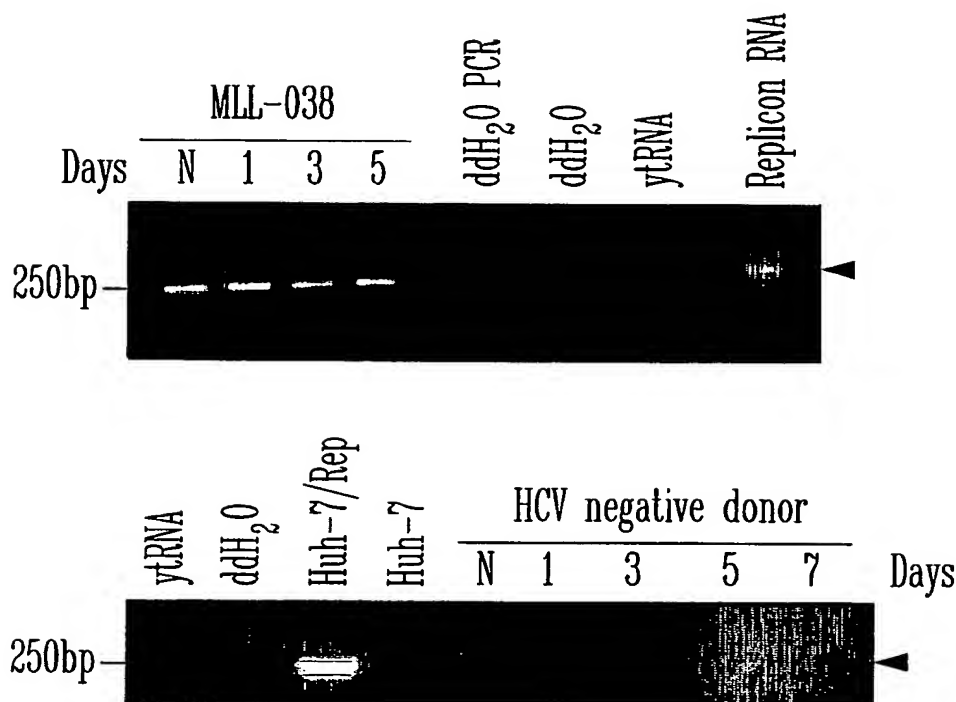


FIG. 23 A

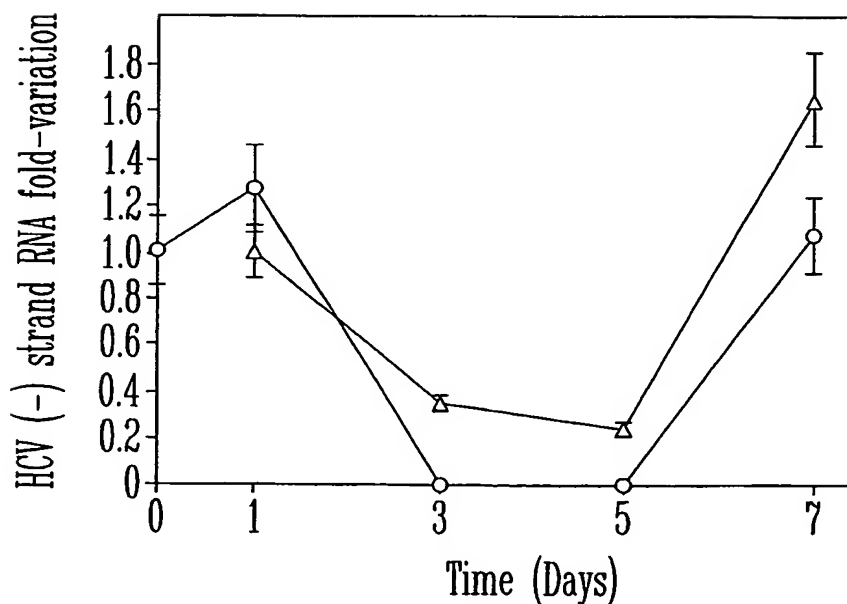


FIG. 23 B

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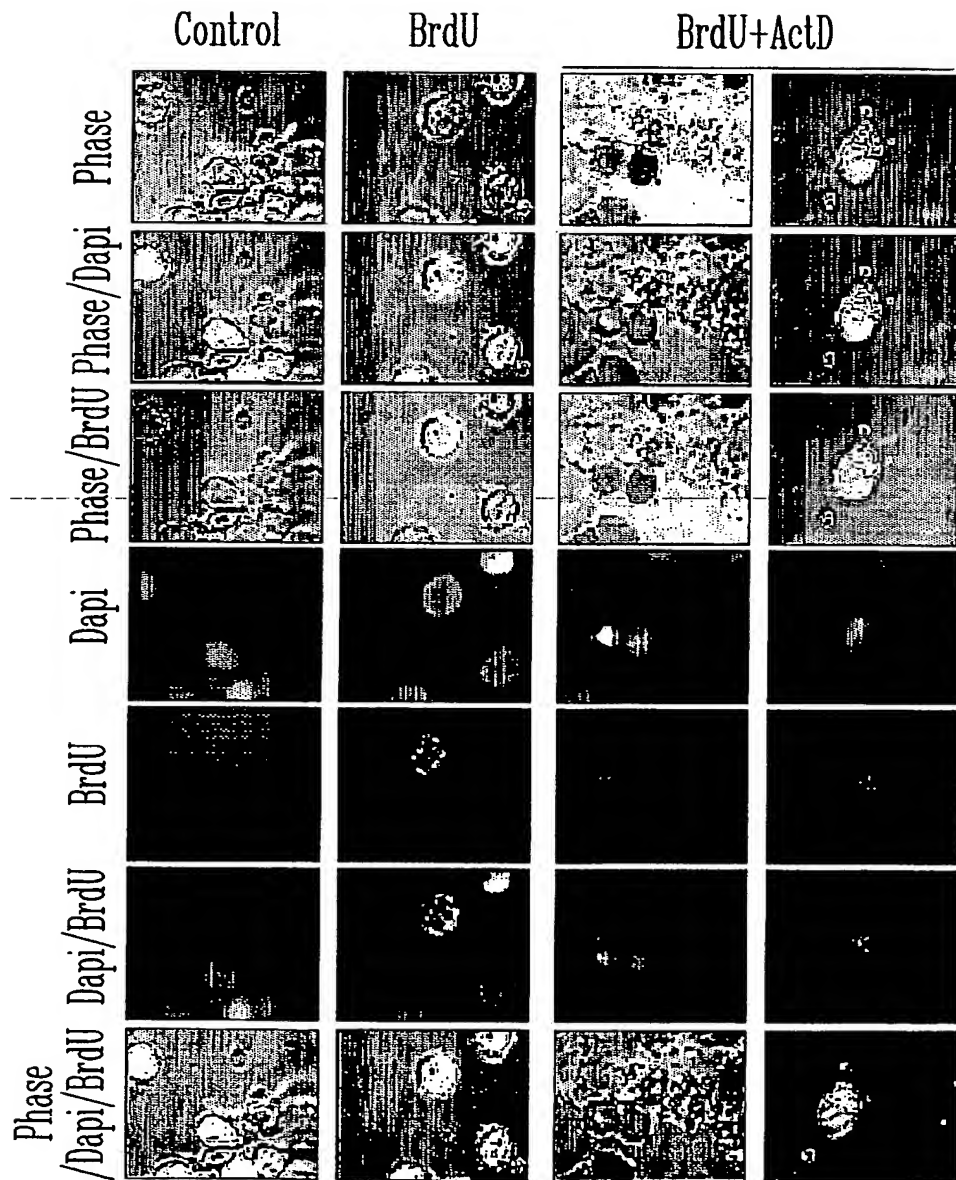


Fig. 23C



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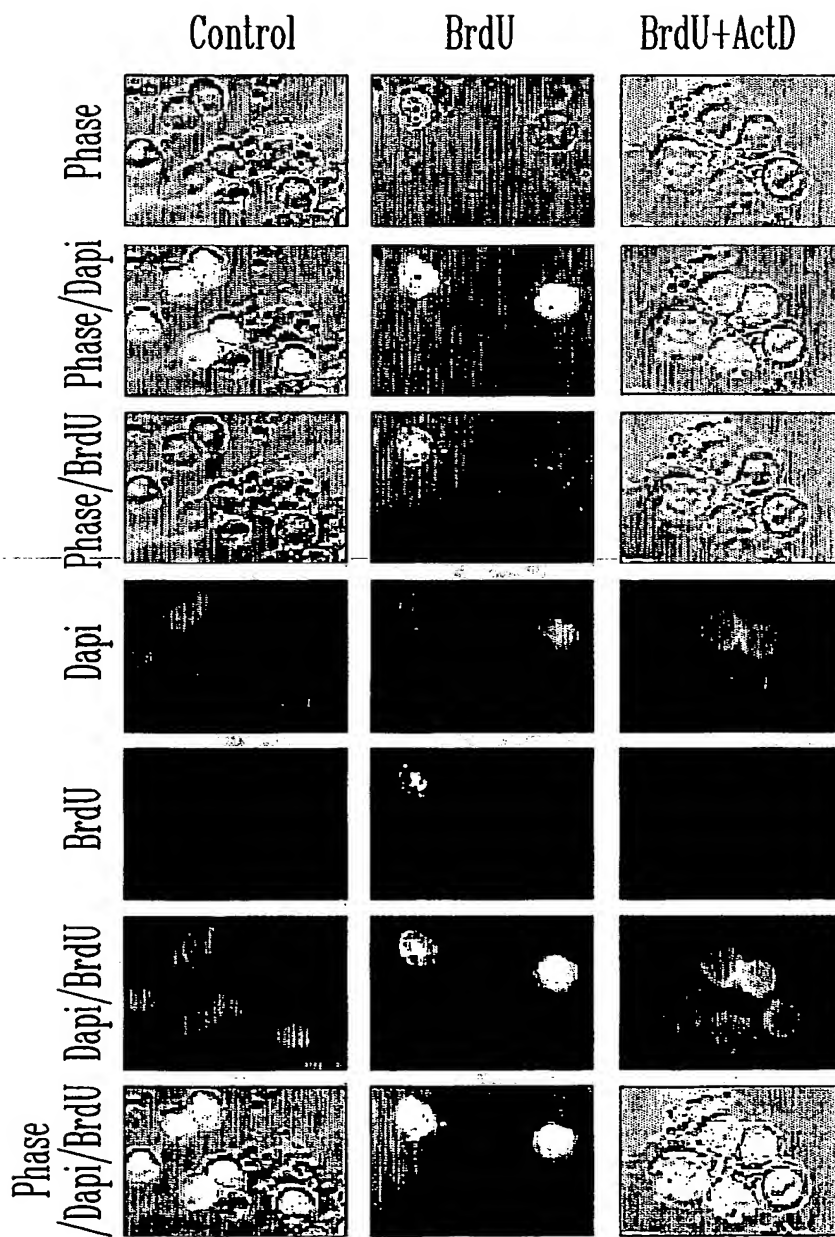
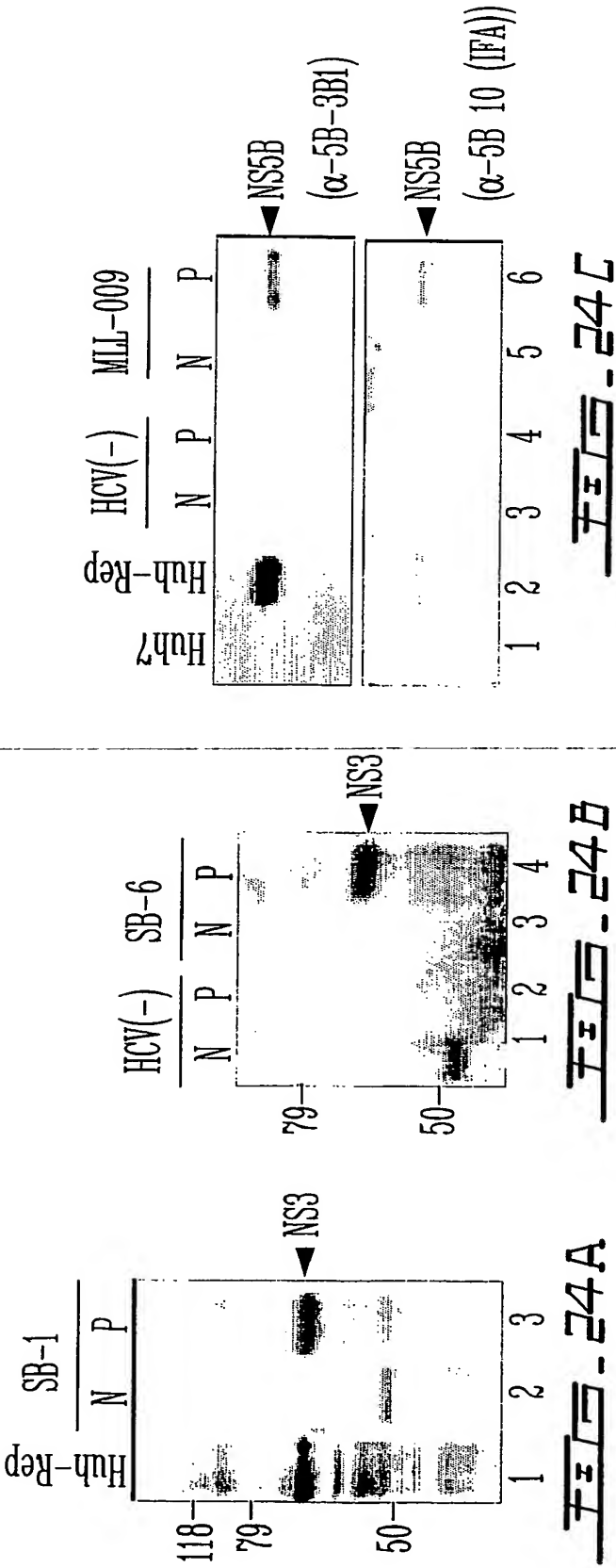
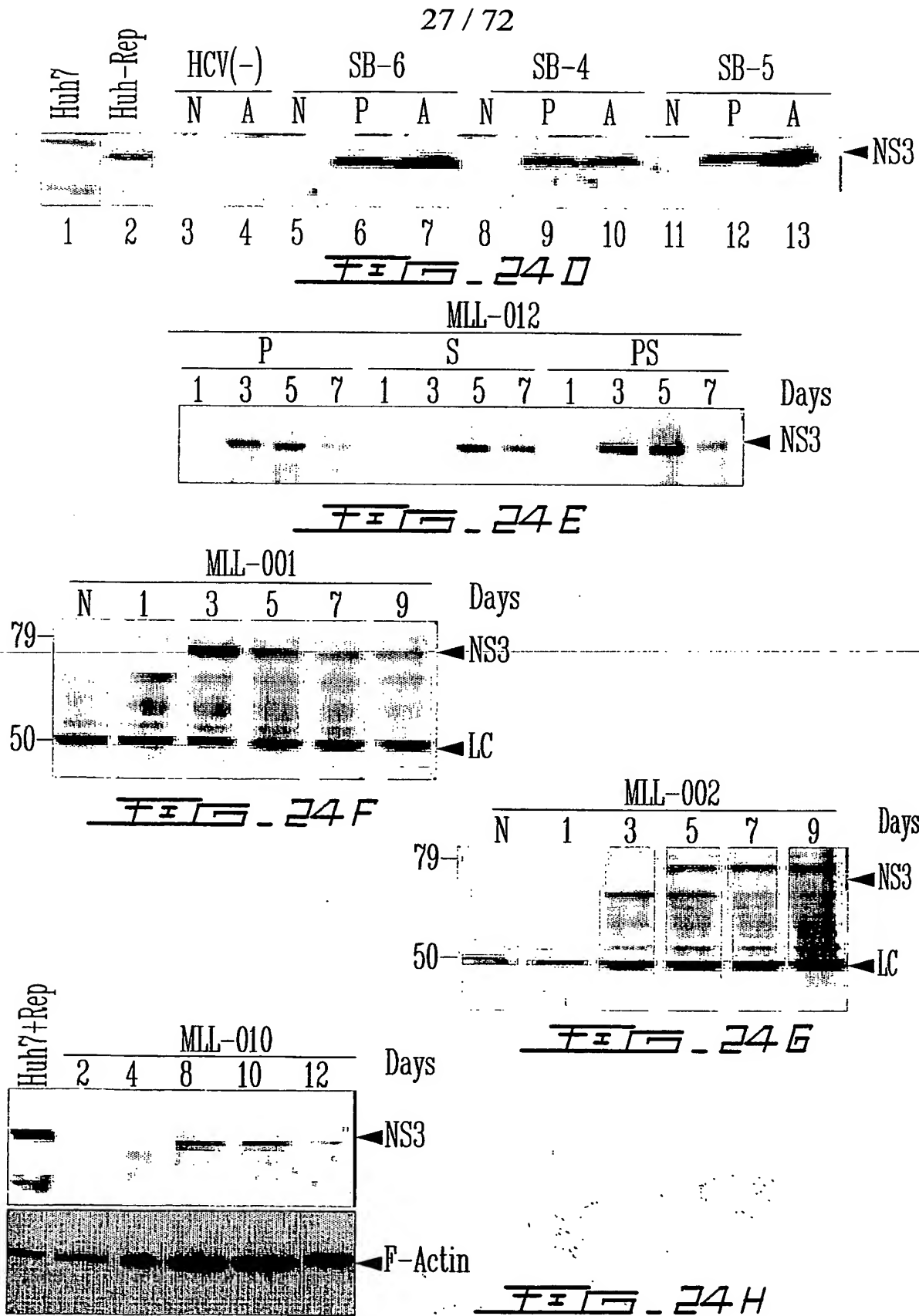


FIG. 23D





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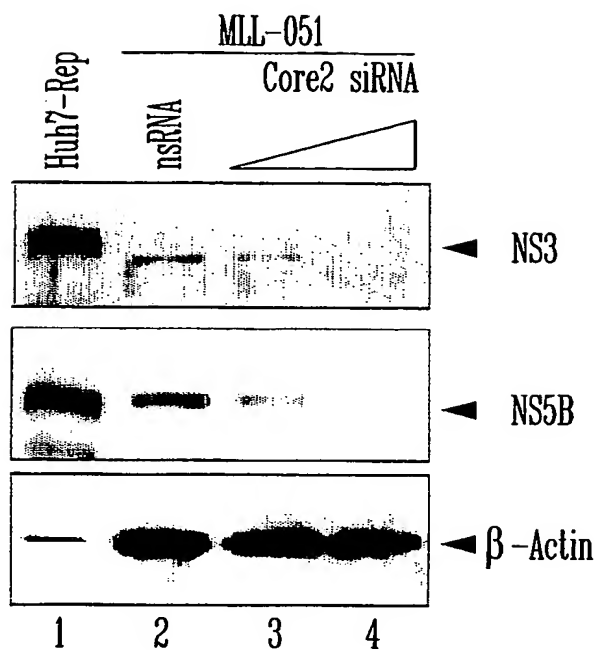


FIG. 24I

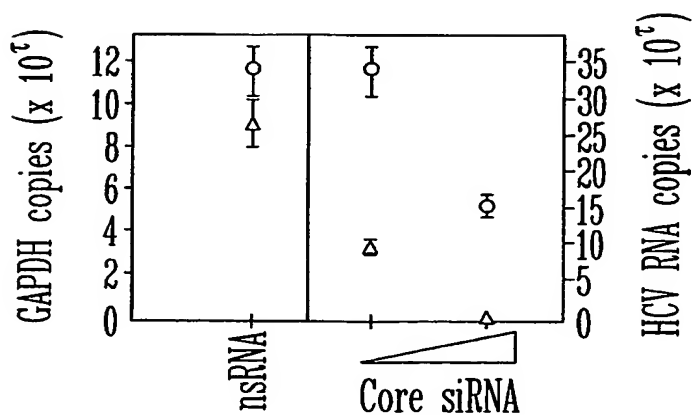


FIG. 24J

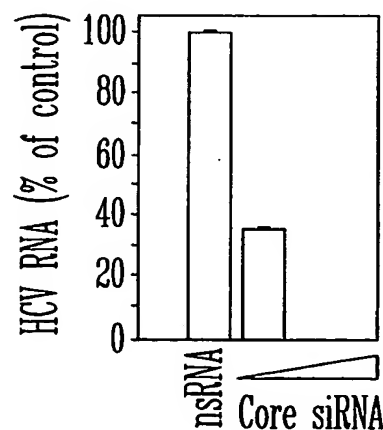


FIG. 24K

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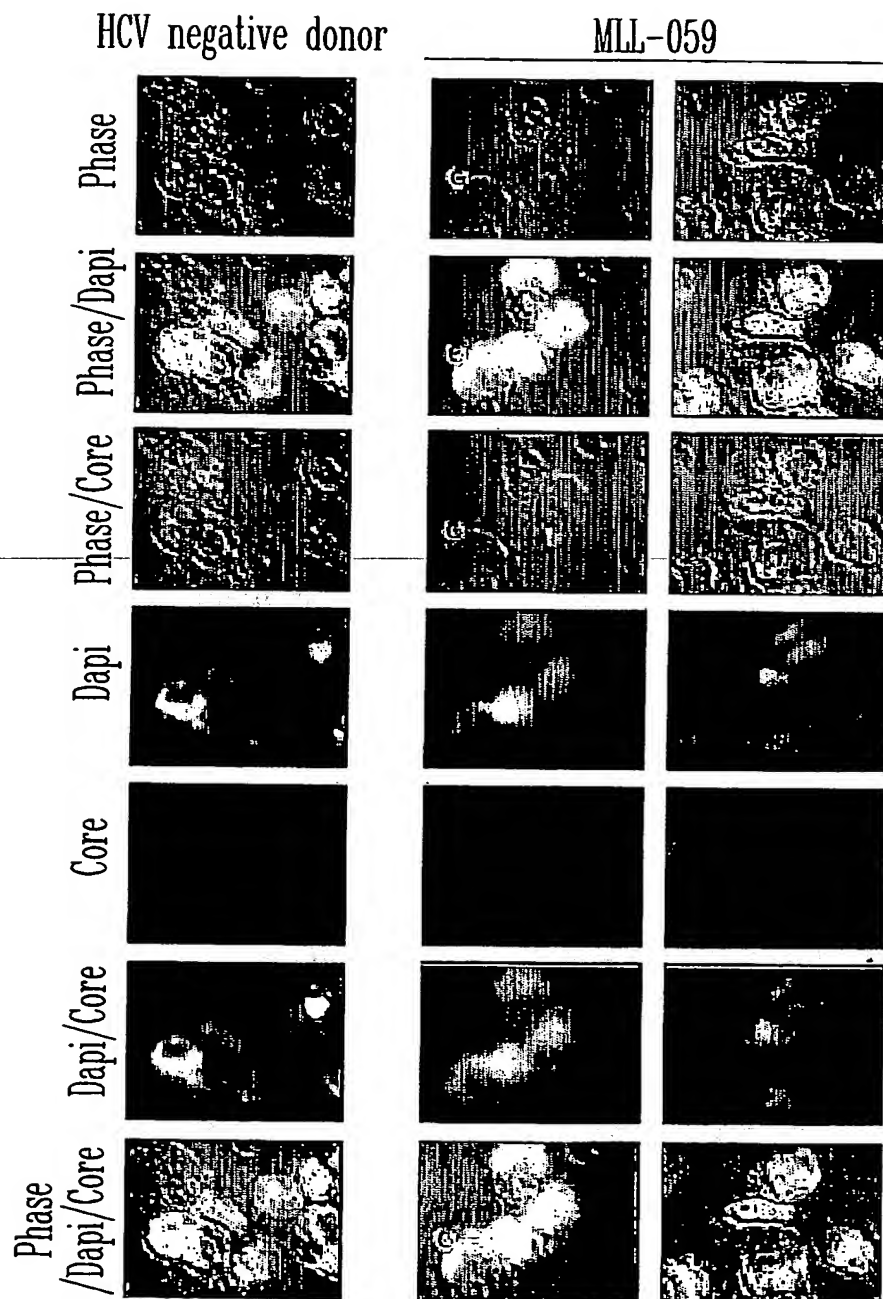


FIG. 25

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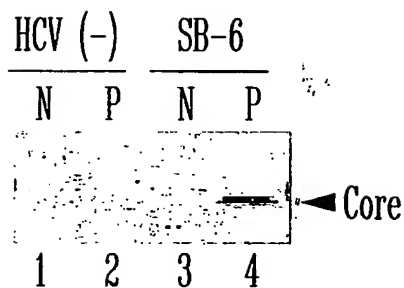


FIG. 26A

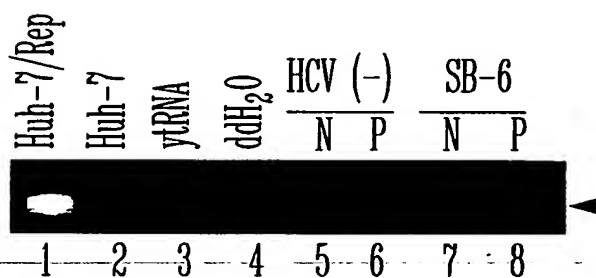
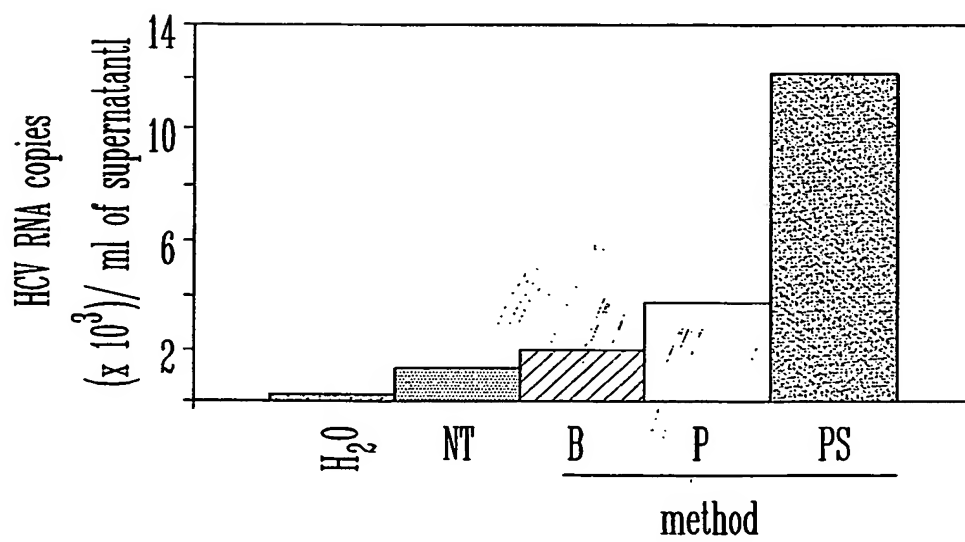


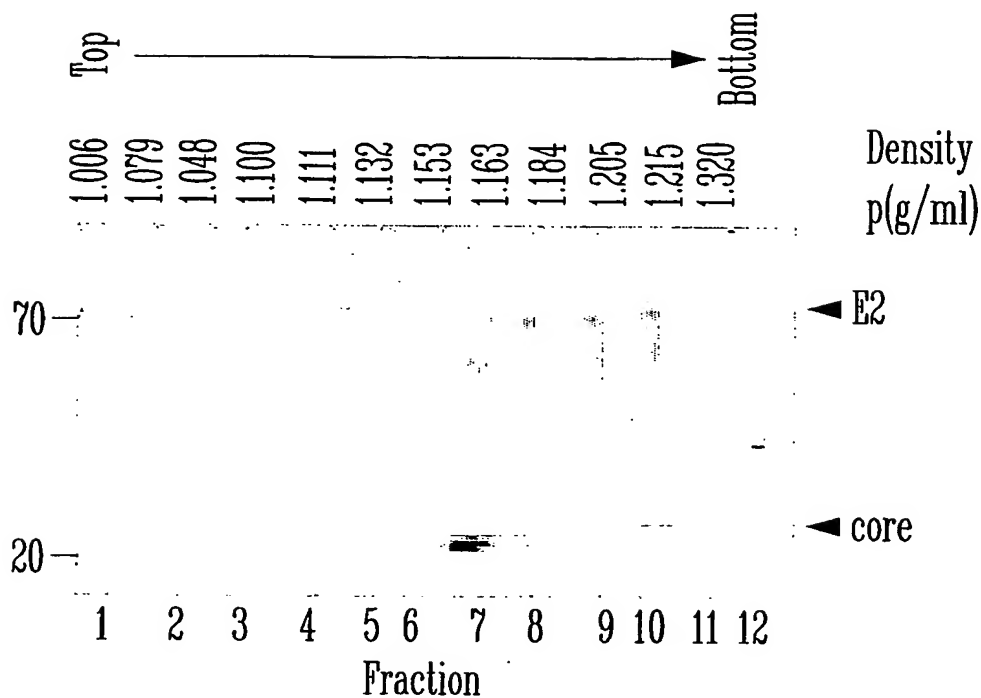
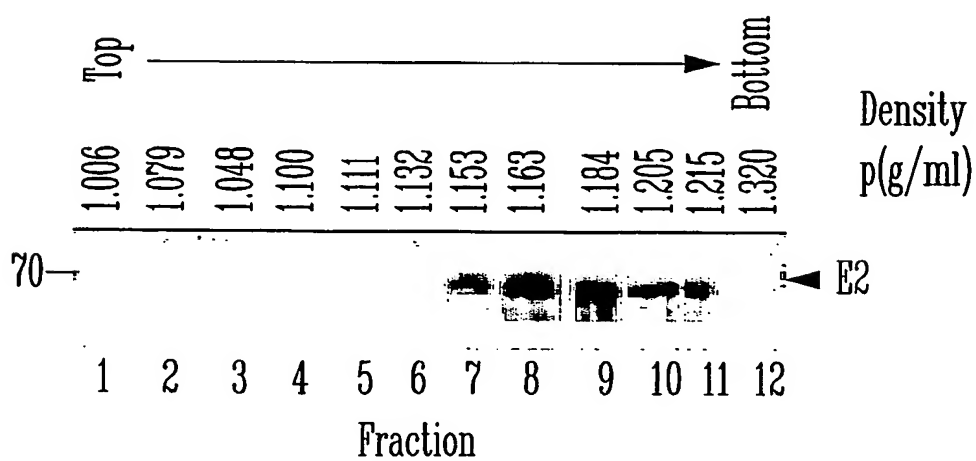
FIG. 26B



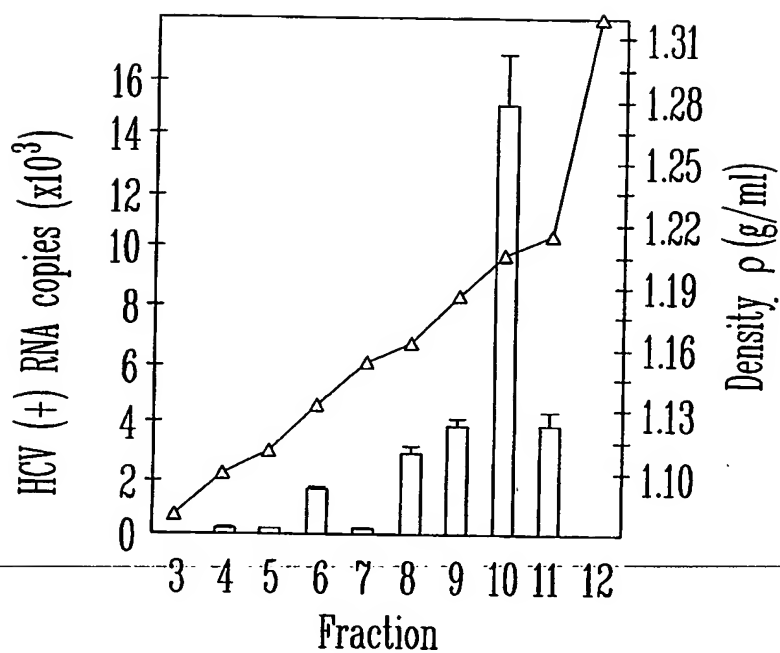
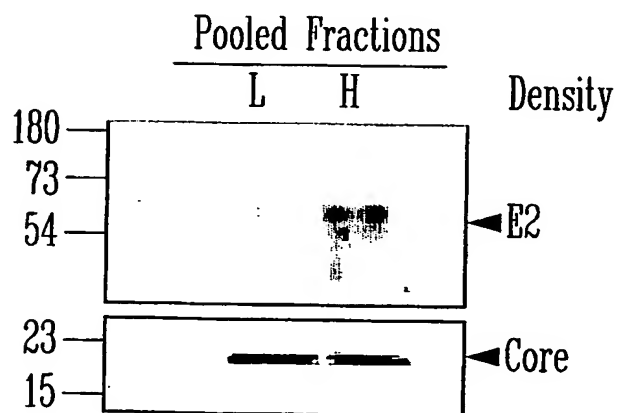
SB-5

FIG. 26C

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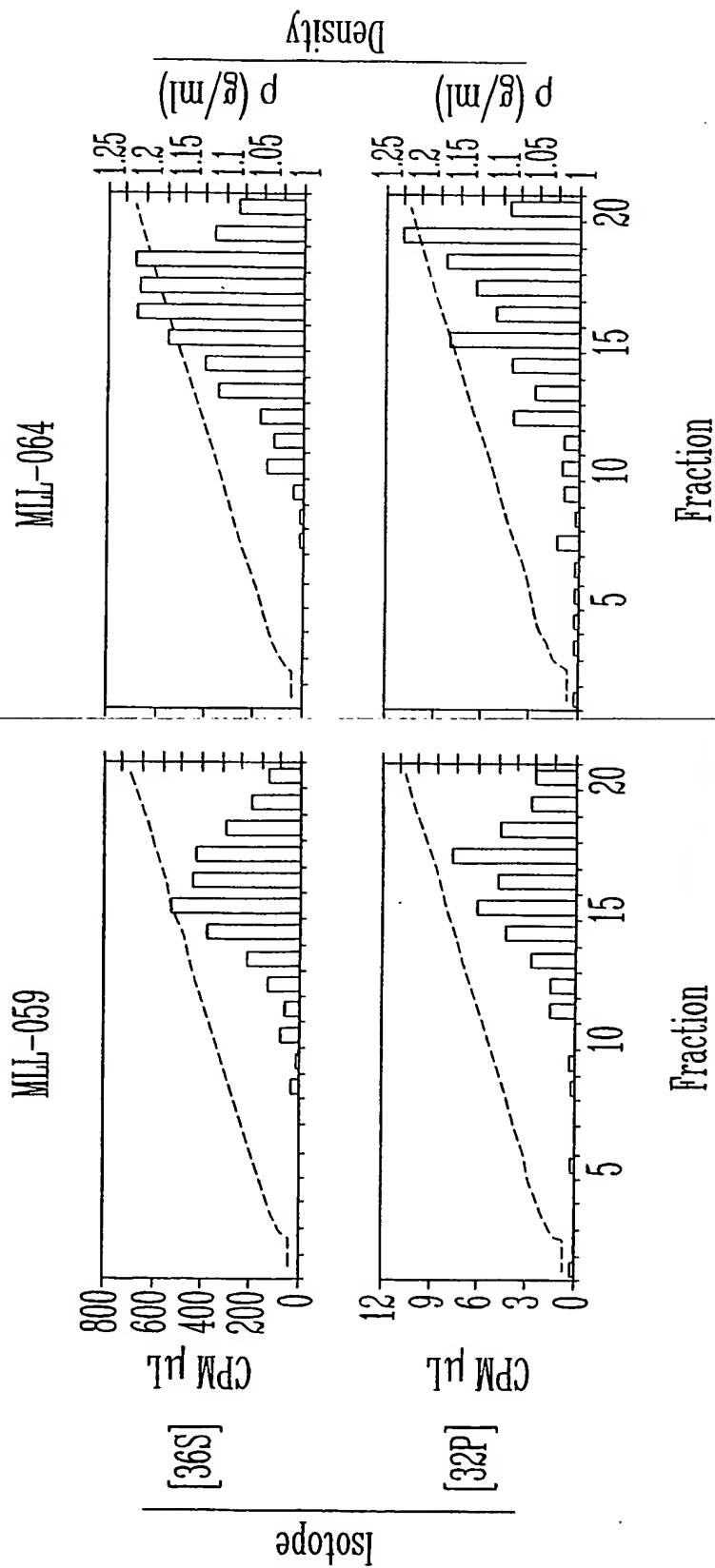
FIG. 260FIG. 26E

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FIG. 26FFIG. 26G

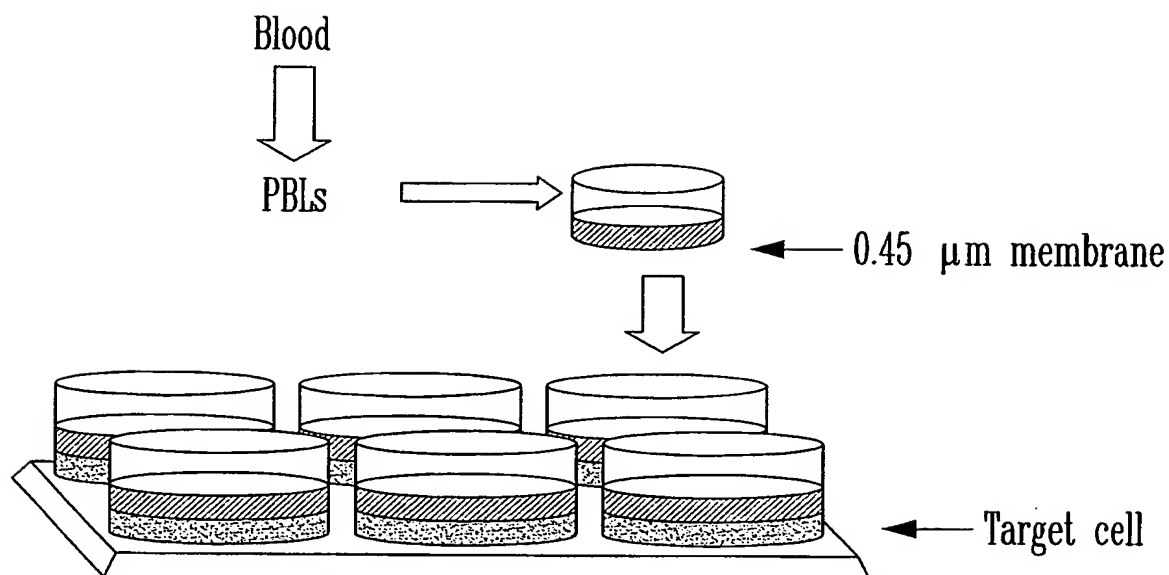
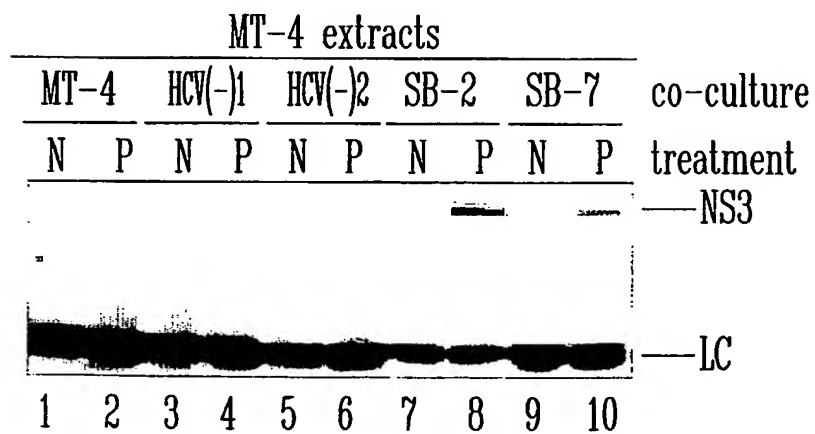


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7-10-2004

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FIG. 27AFIG. 27B

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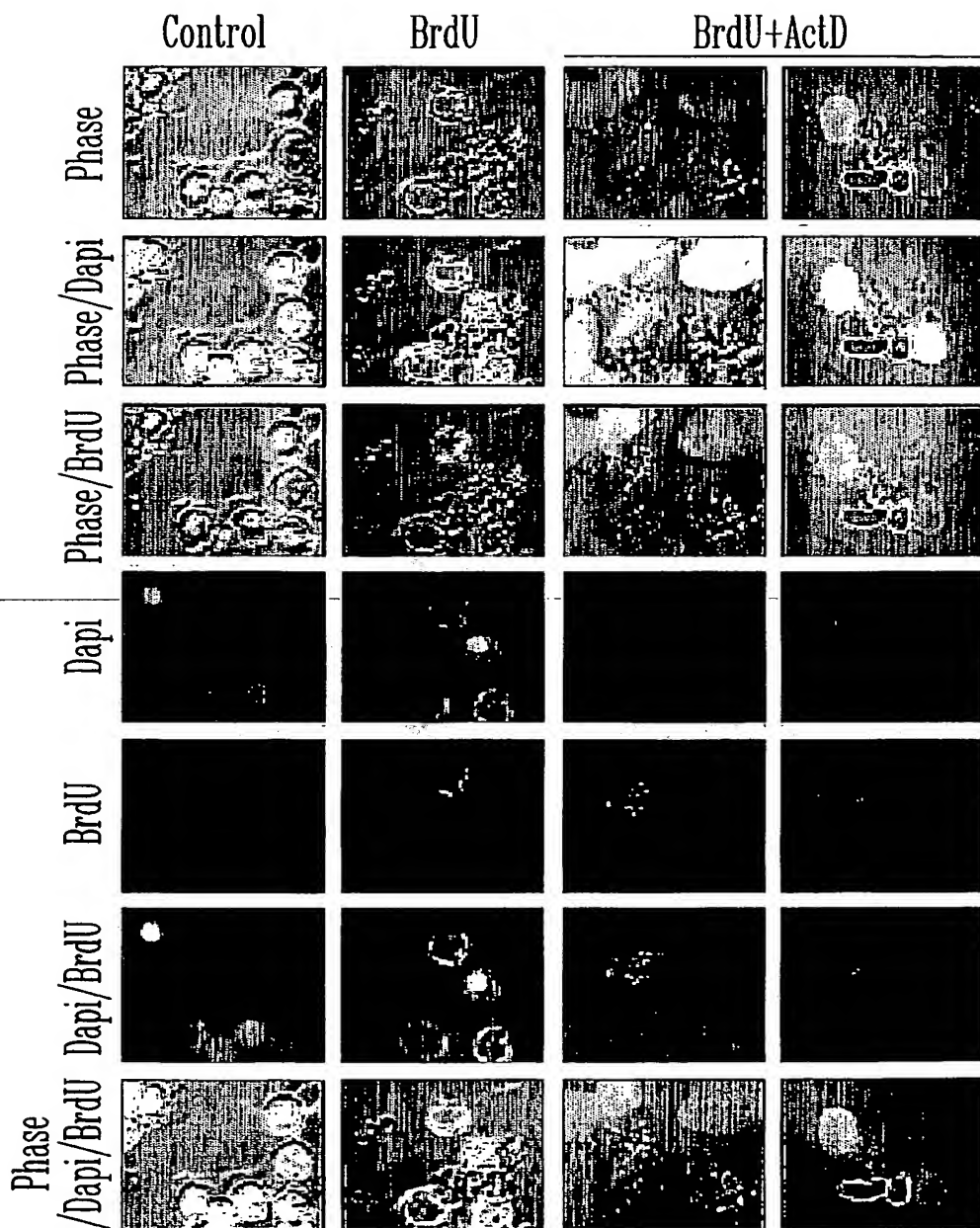
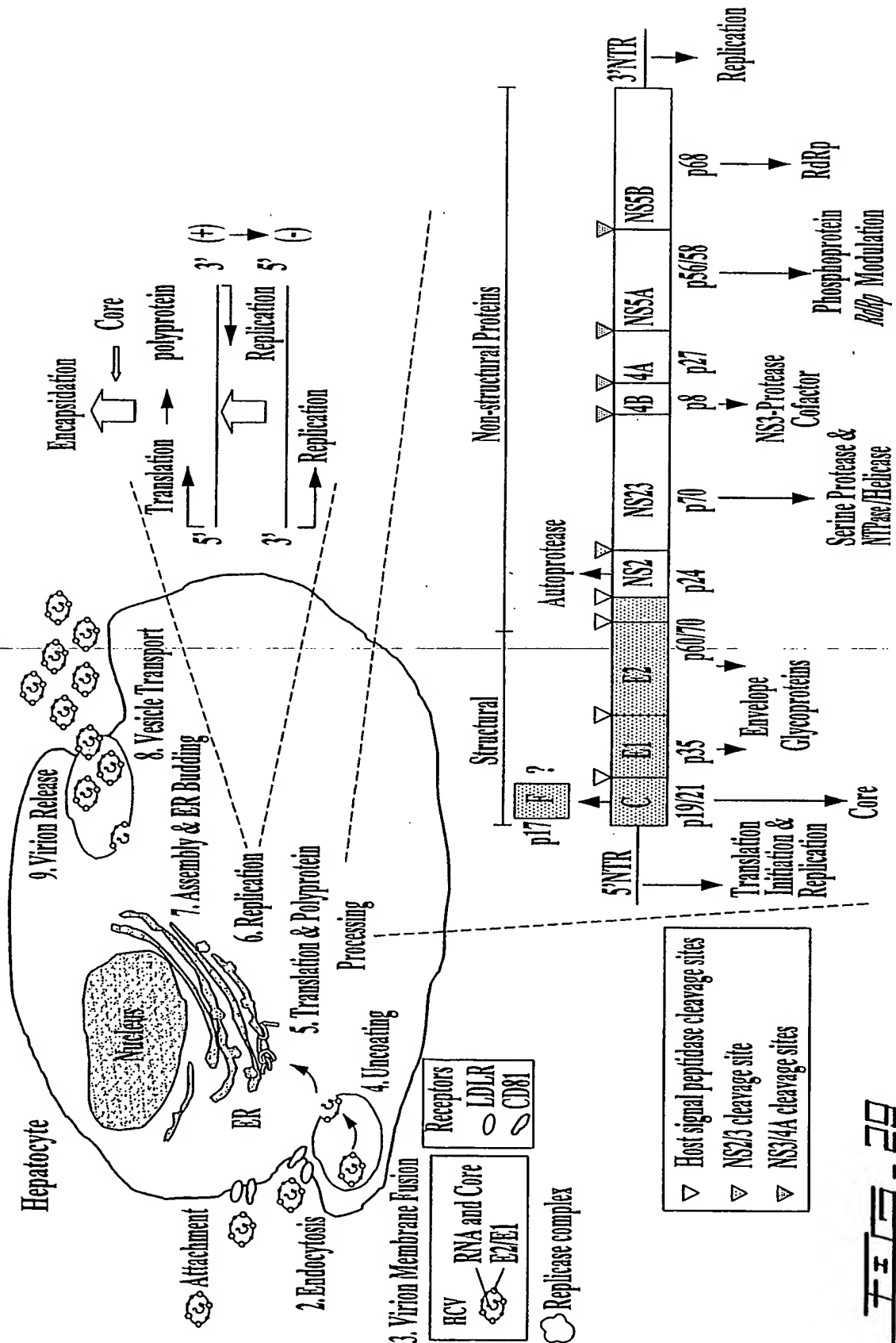


FIG. 2B

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# HCV Replication Cycle



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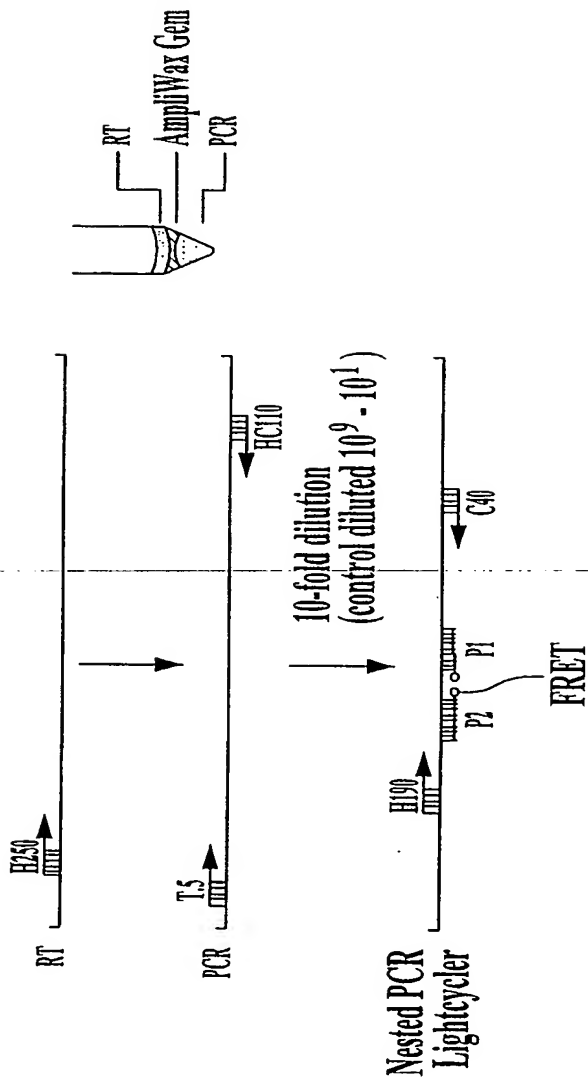
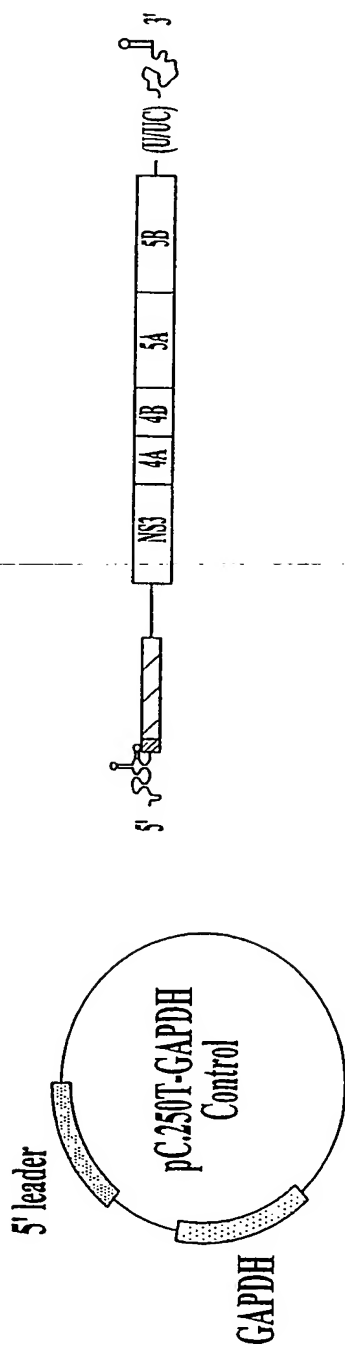


FIG. 30A

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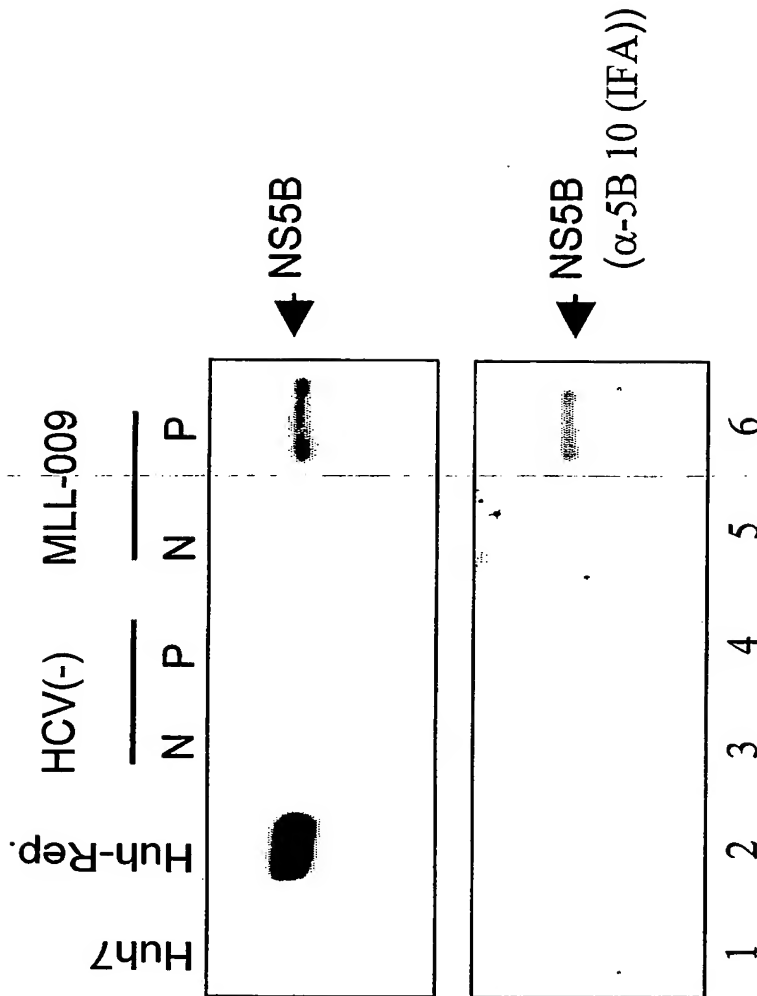
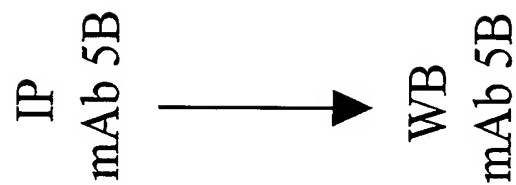


Fig. 30B

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MLL031 +MLL 032  
HCV(+)

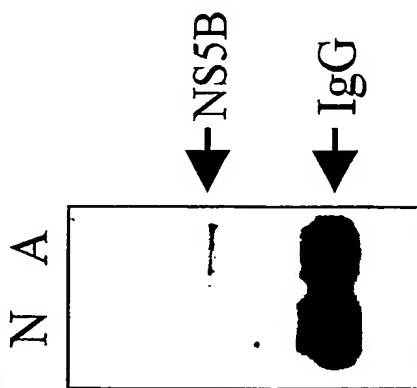
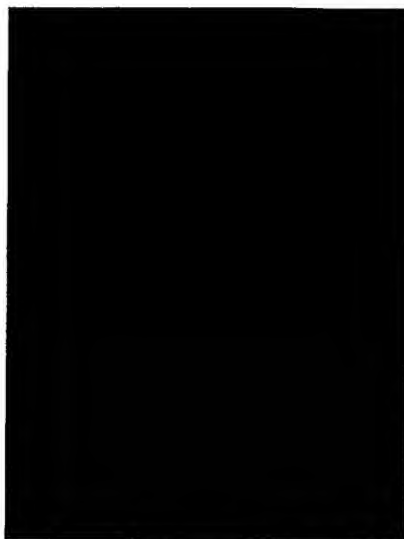


FIG. 31

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Anti-Core



Dapi



Phase



Phase/Dapi/Anti-Core



Dapi/Anti-Core

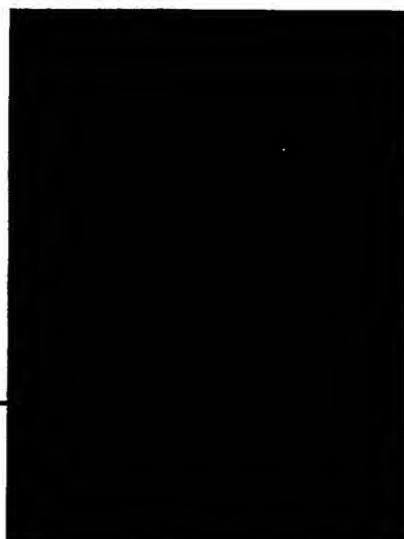
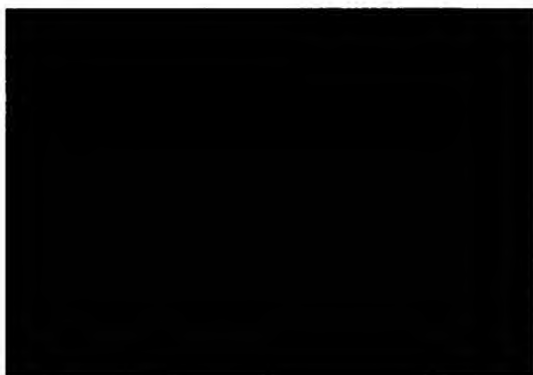


FIG. 32



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Anti-Core



Dapi



Phase



Phase/Dapi/Anti-Core



Dapi/Anti-Core



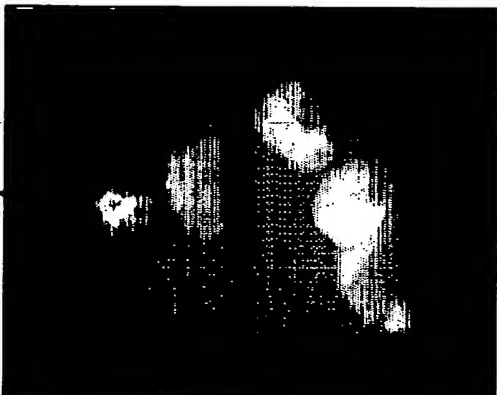
FILE-33

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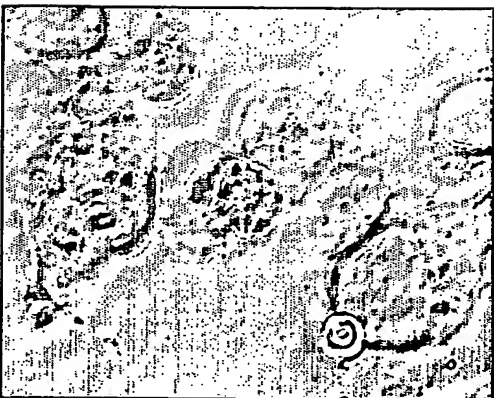
Anti-Core



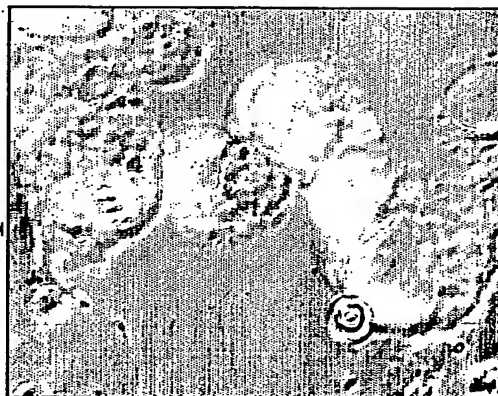
Dapi



Phase



Phase/Dapi/Anti-Core



Dapi/Anti-Core



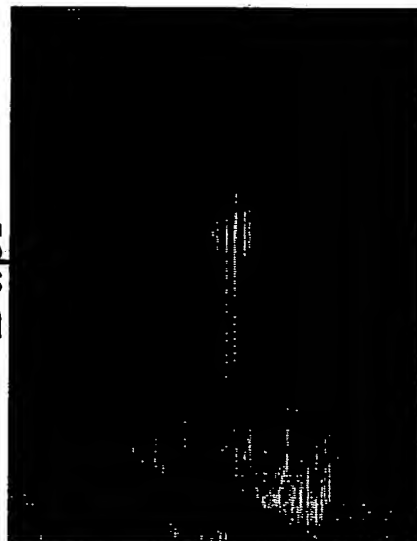
FIG. 34

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Phase



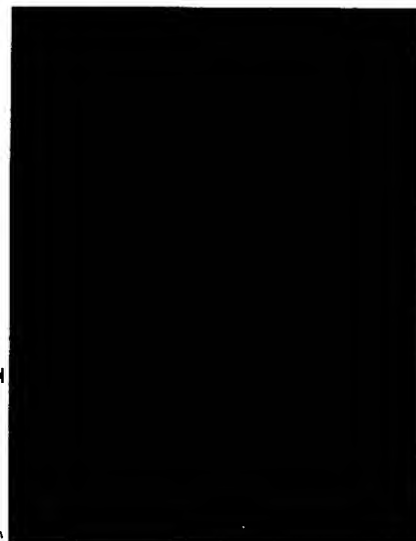
Dapi



Anti-Core



Dapi/Anti-Core



Phase/Dapi/Anti-Core

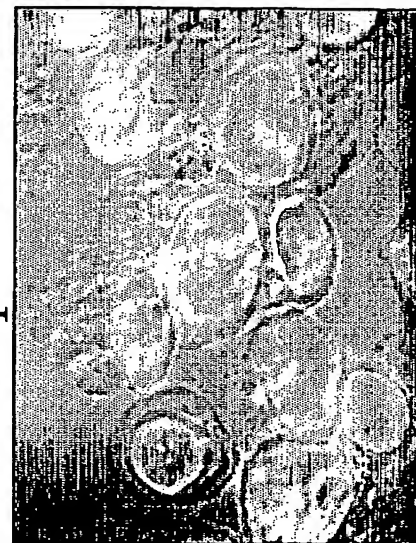
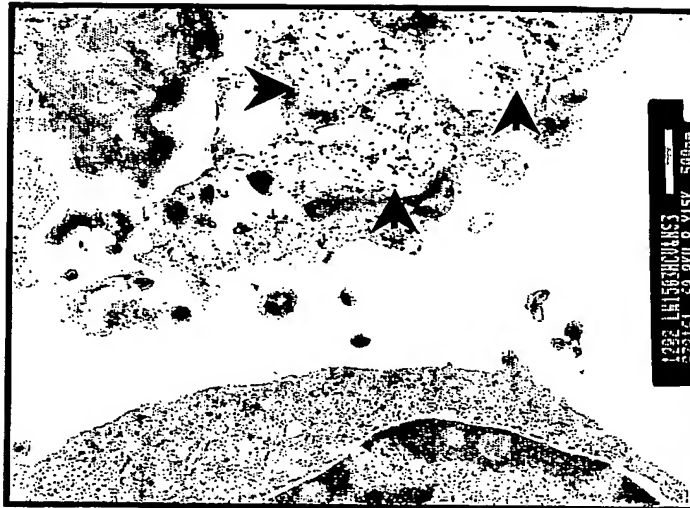


FIG. 35

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FEB-36C



FEB-36B



FEB-36A

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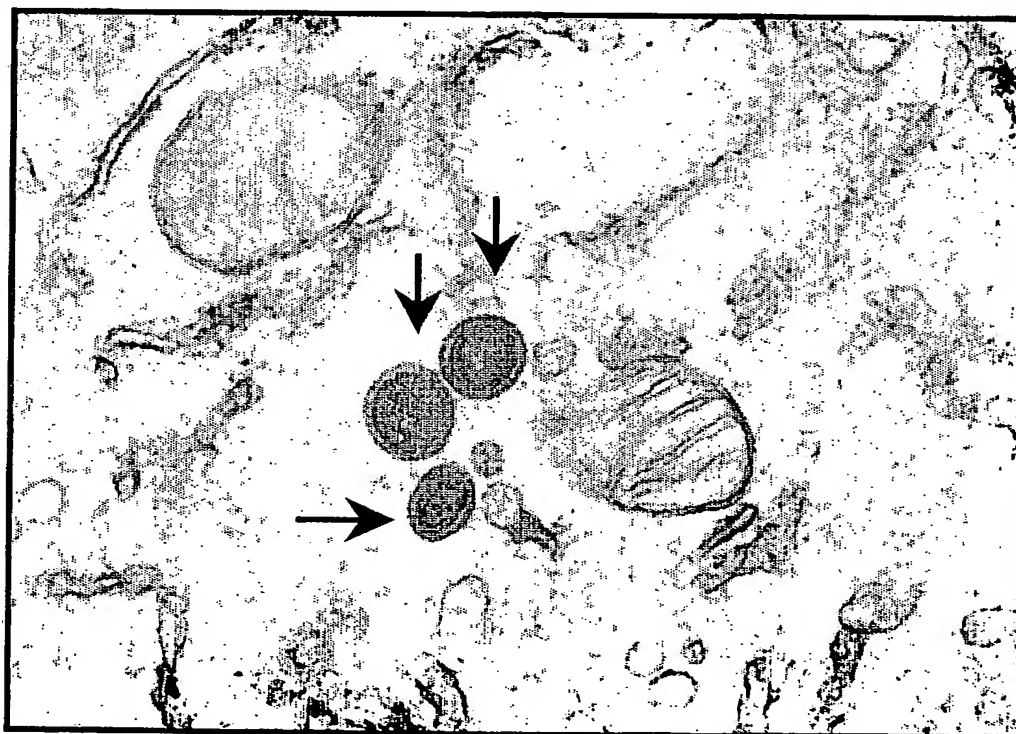
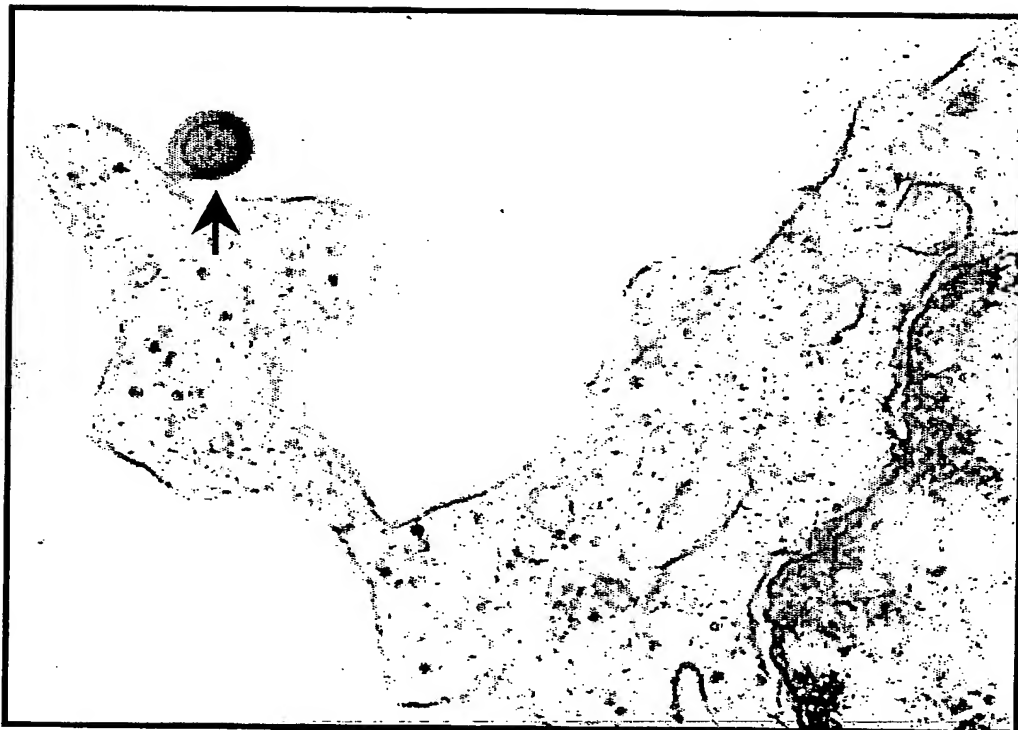


FIG. 37

Virus partial purification.

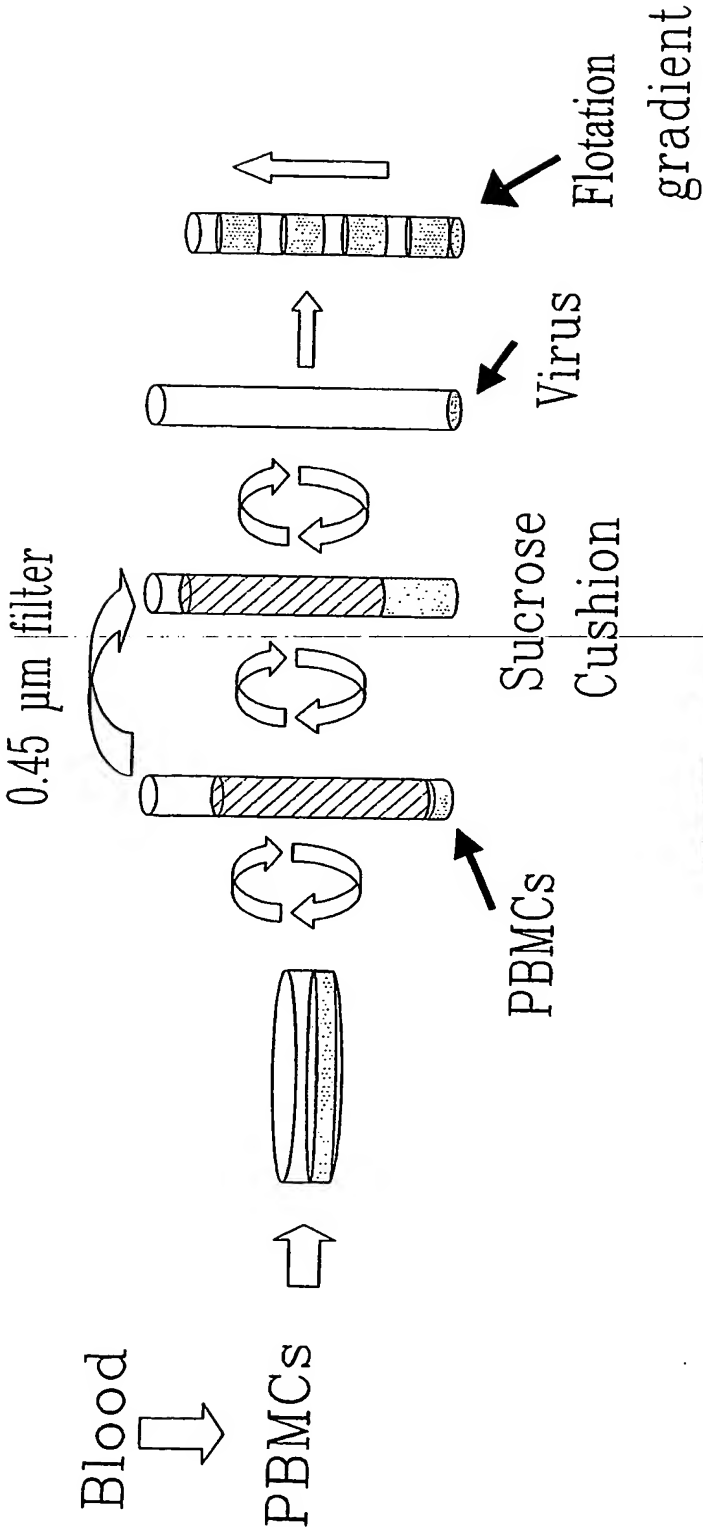


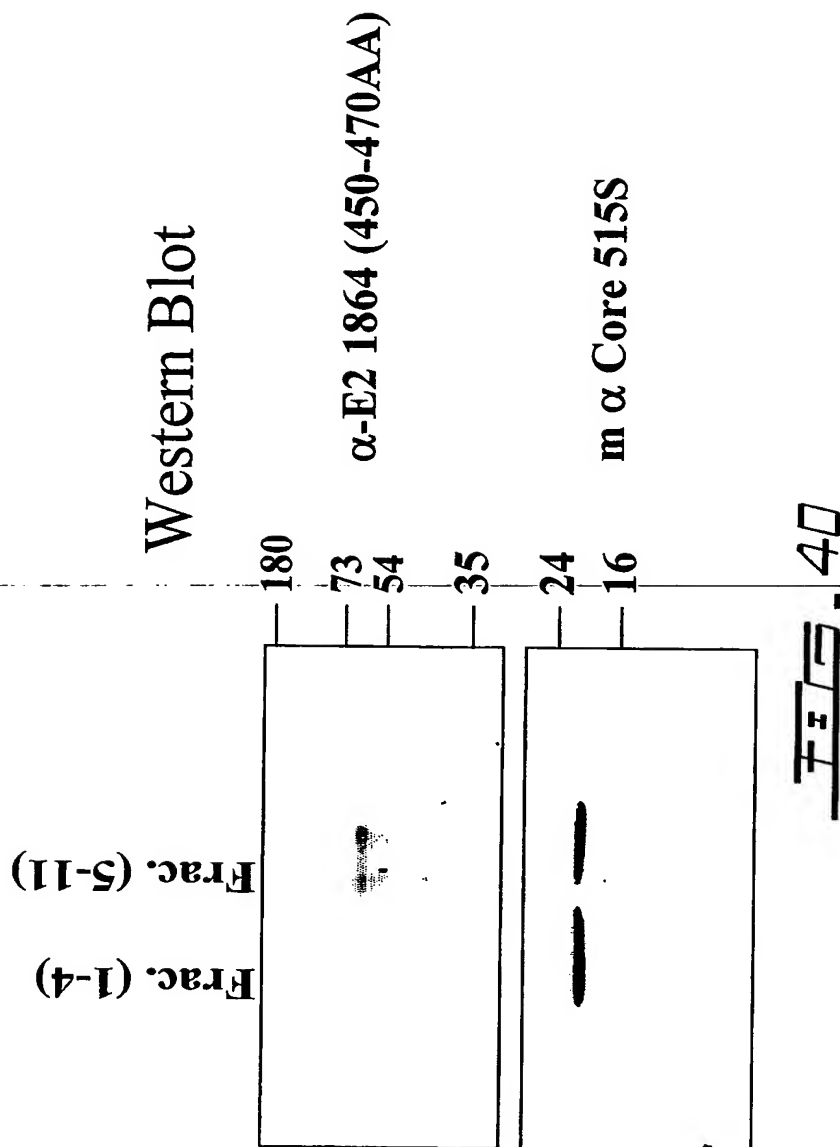
FIG. 3B

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Density Range (g/ml)	Source	Reference
1.15-1.20	HCV-LP in VSV vector	J.Virol (2002) 76, 12325.
1.14-1.18	HCV-LP in insect cells	J. Virol (1998) 72, 3827.
1.12-1.17	Plasma chimps	J. Gen.Virol (1994) 75, 1755
1.09-1.21	Plasma chimps	J.Med.Virol (1991), 34, 206.
1.13-1.17	Plasma chimps	J.Virol (1993) 67, 1953
1.063-1.21	Serum infected donors	J Med Virol (2002) 68, 335
1.11-1.215	HCV(+) PBMCs	-----

7370.39

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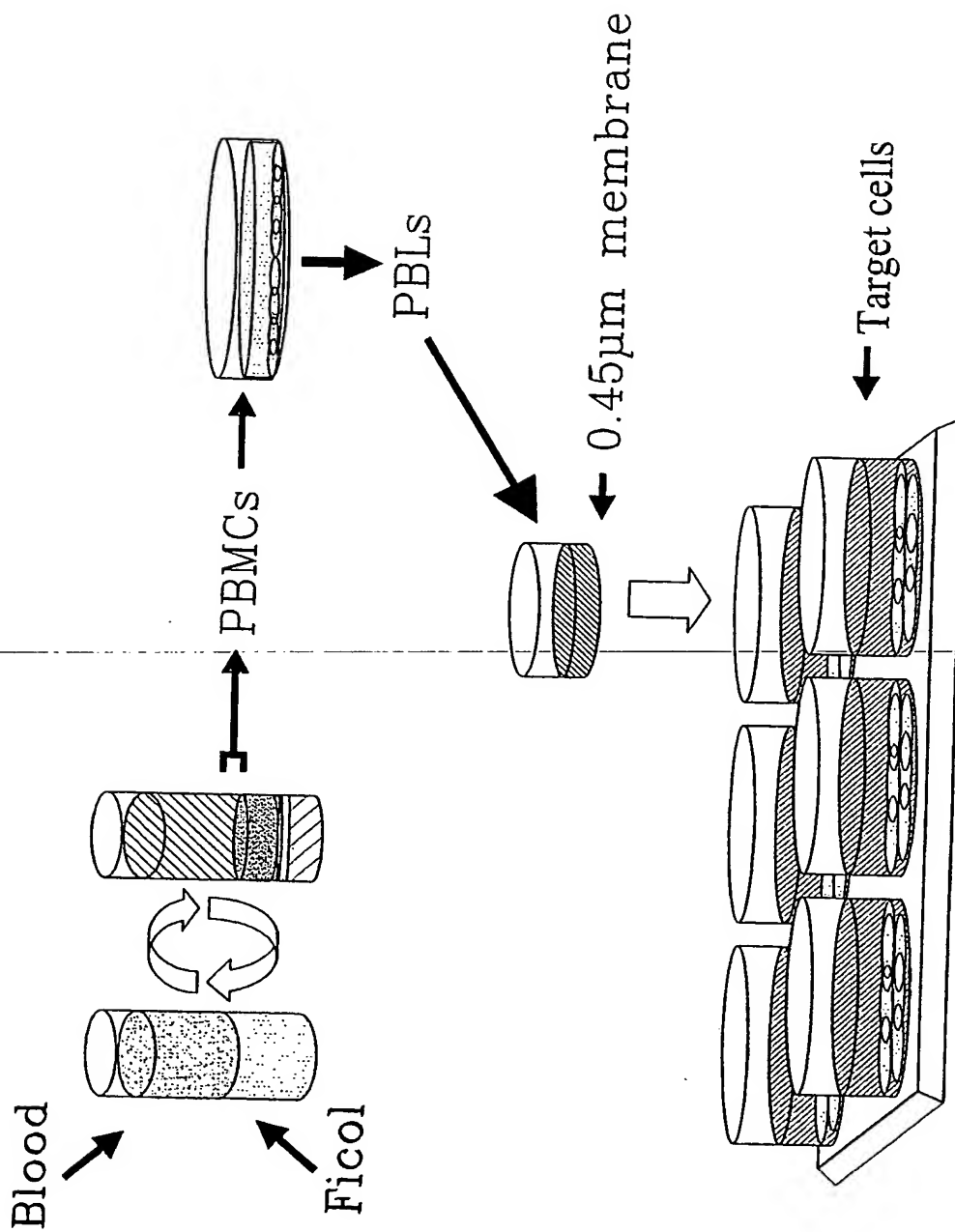
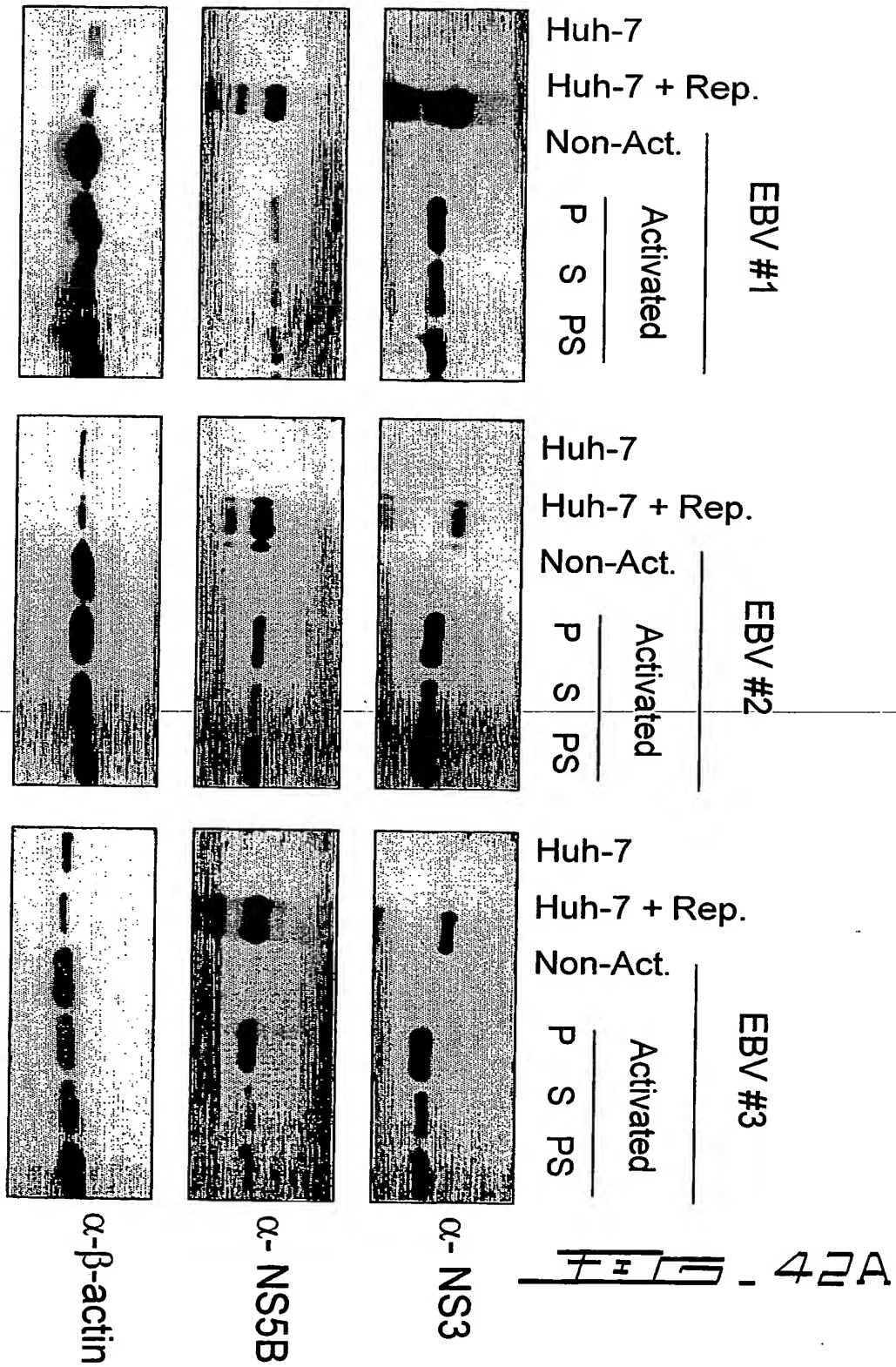


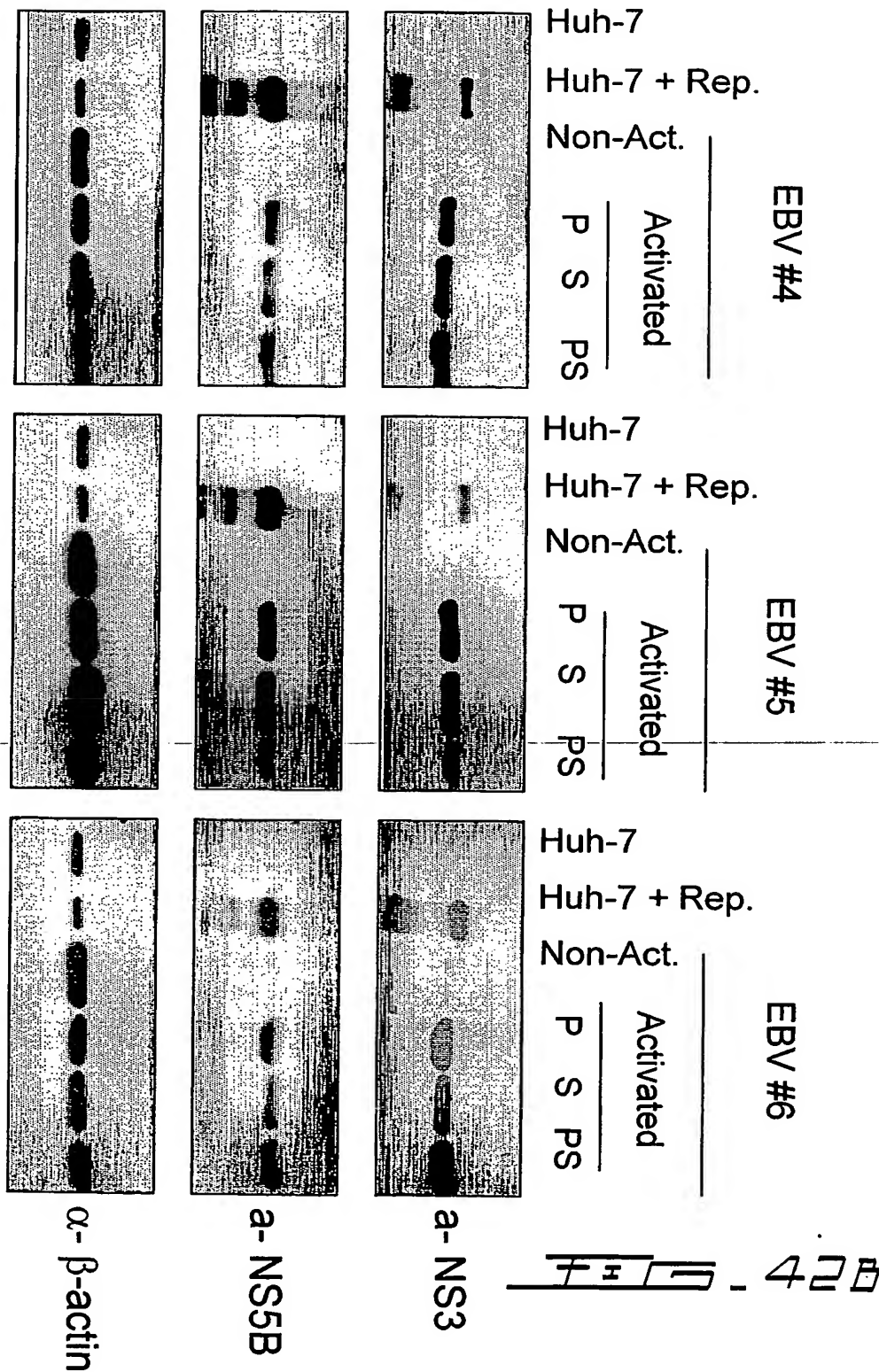
Fig. 41

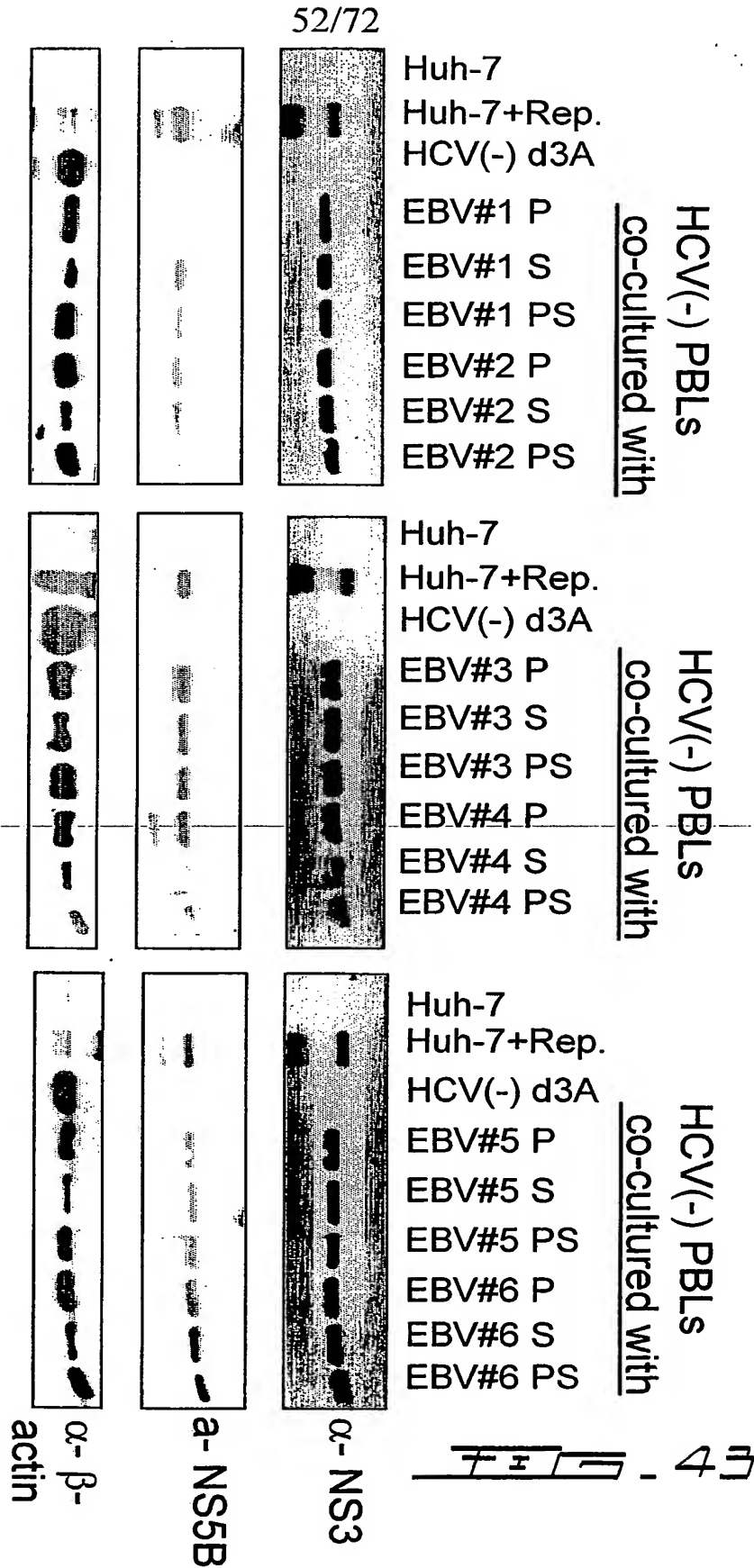
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42A

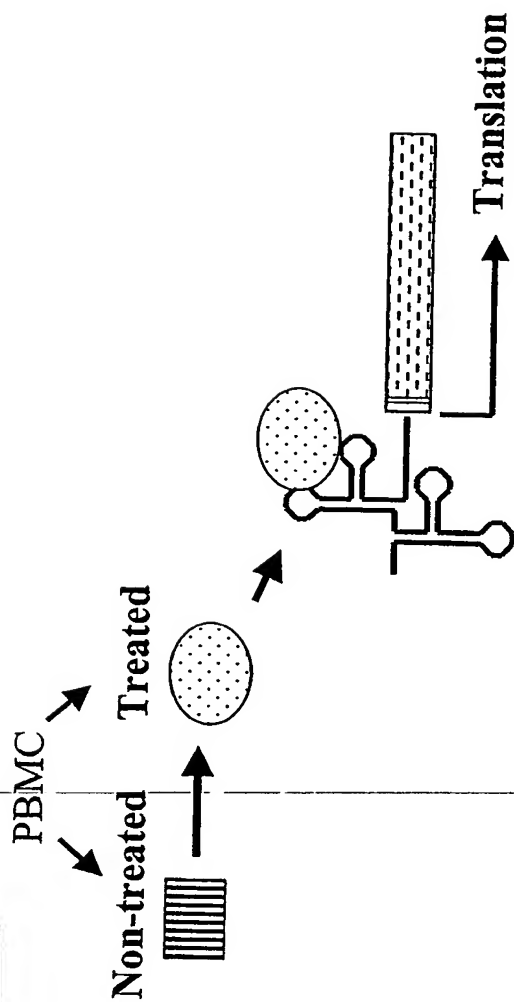
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# I- Translation Activator.



# II- Translation inhibitor.

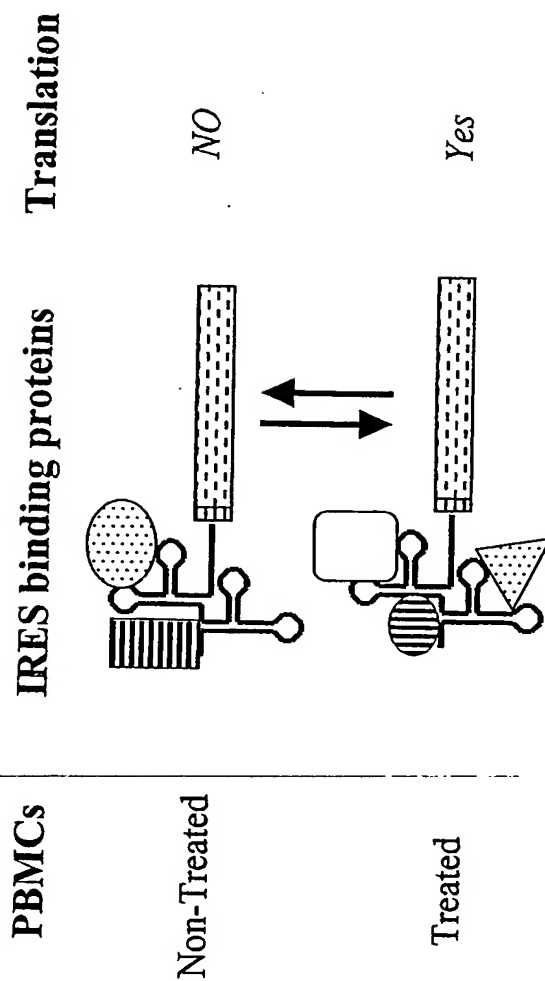
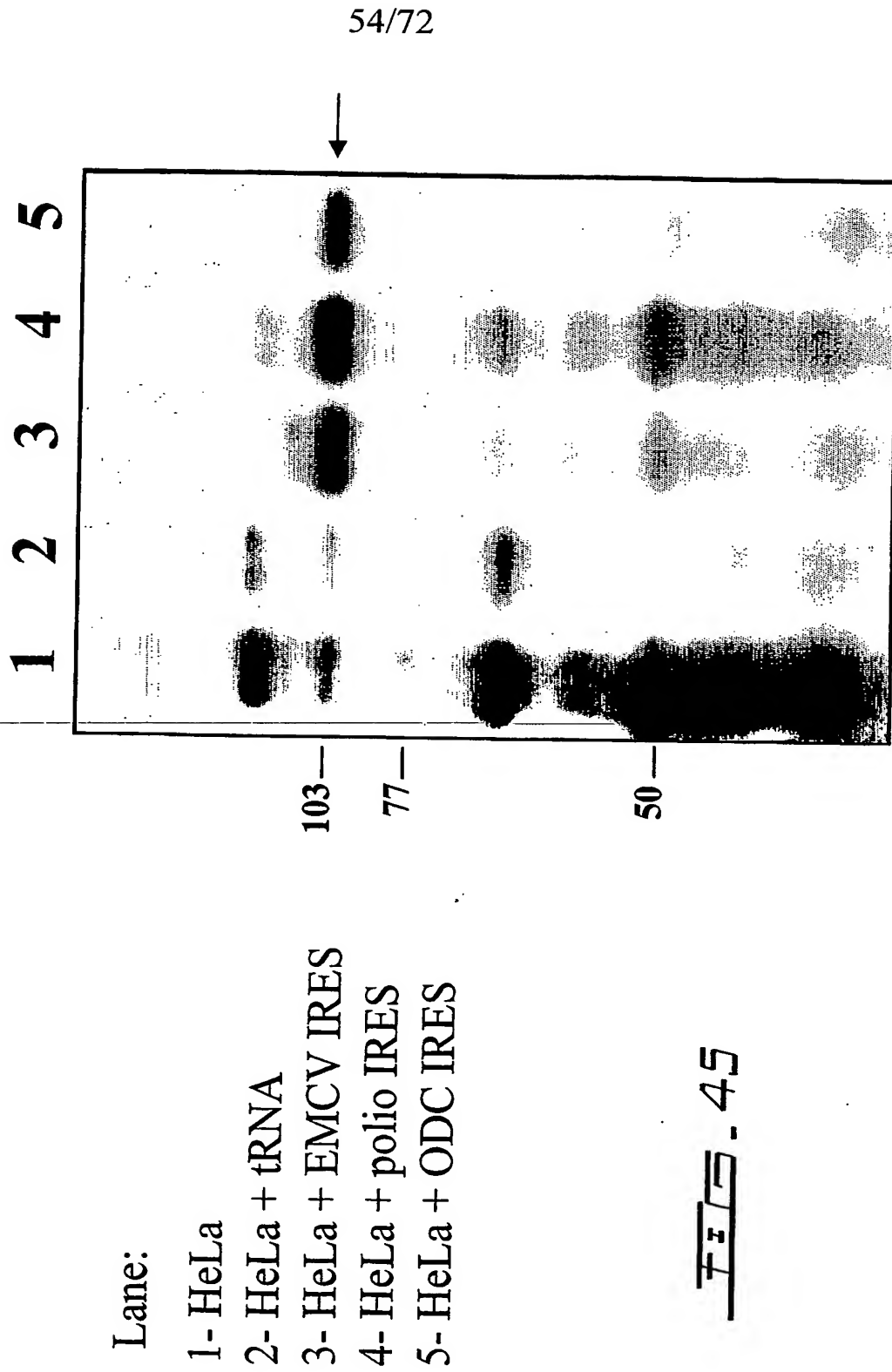
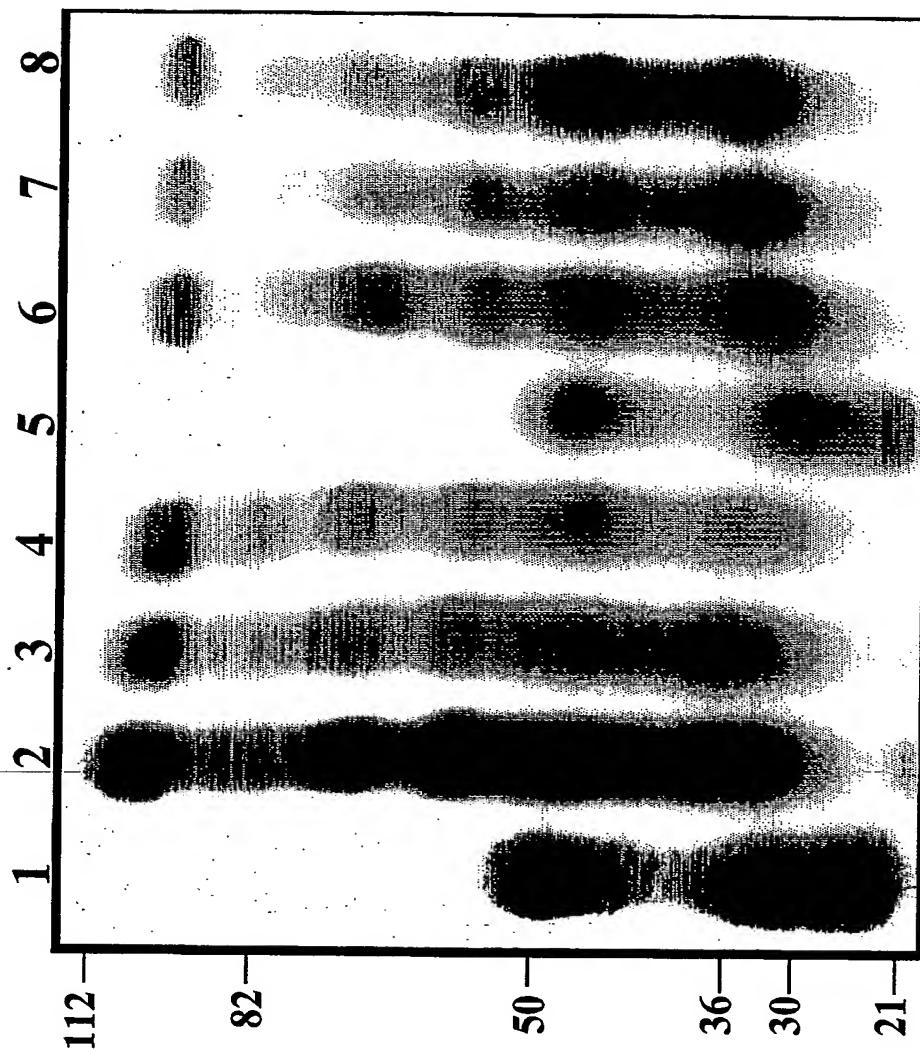


Fig. 44



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Lane:

1- PBMCs NT

2- PBMCs treatment 1

3- PBMCs treatment 2

4- PBMCs treatment

2+DEVA

5- PBMCs NT + HIV

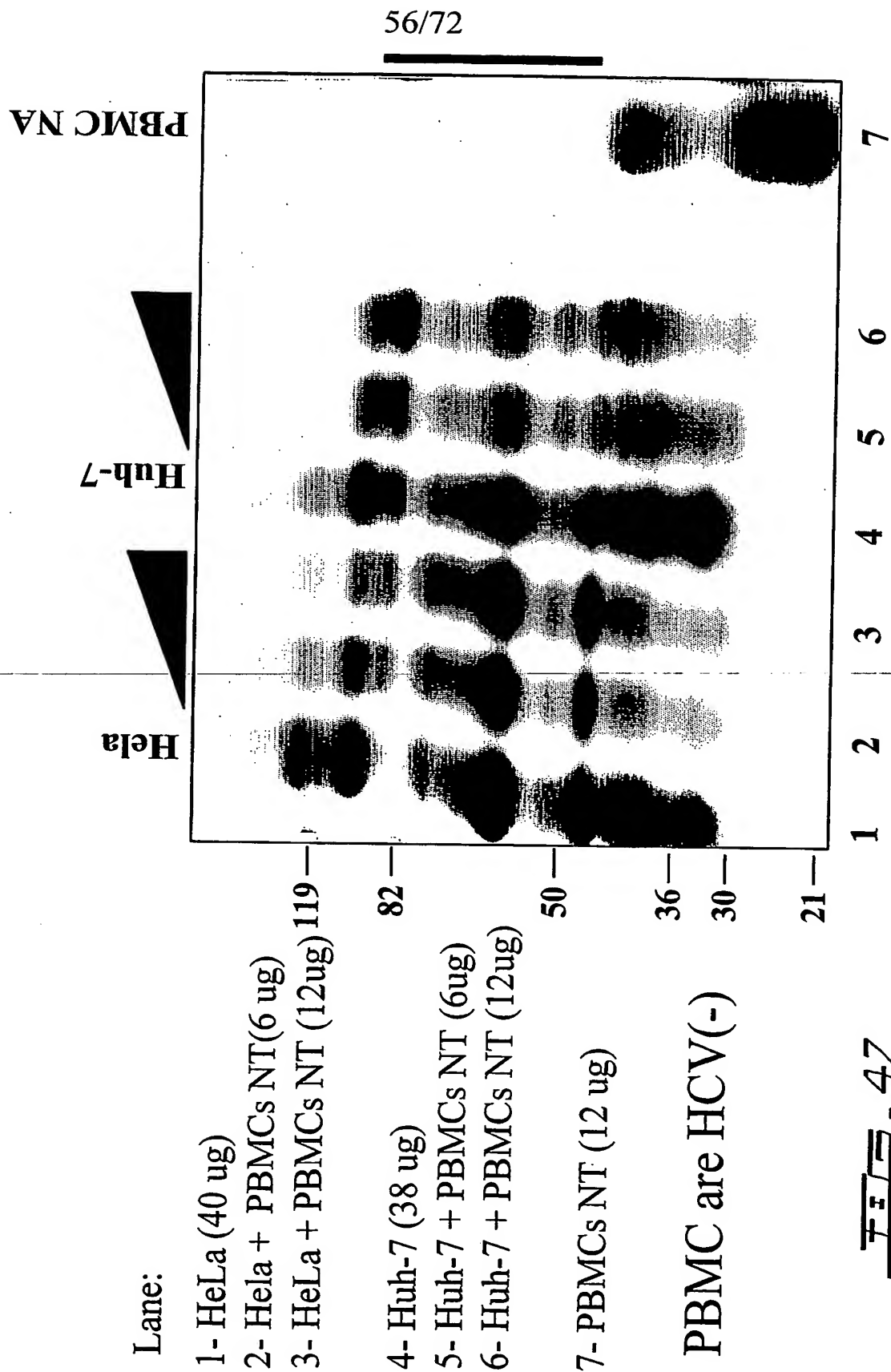
6- PBMCs treatment 1 +HIV

7- PBMCs treatment 2 +HIV

8- PBMCs treatment 2

+DEVA+HIV

Fig. 45





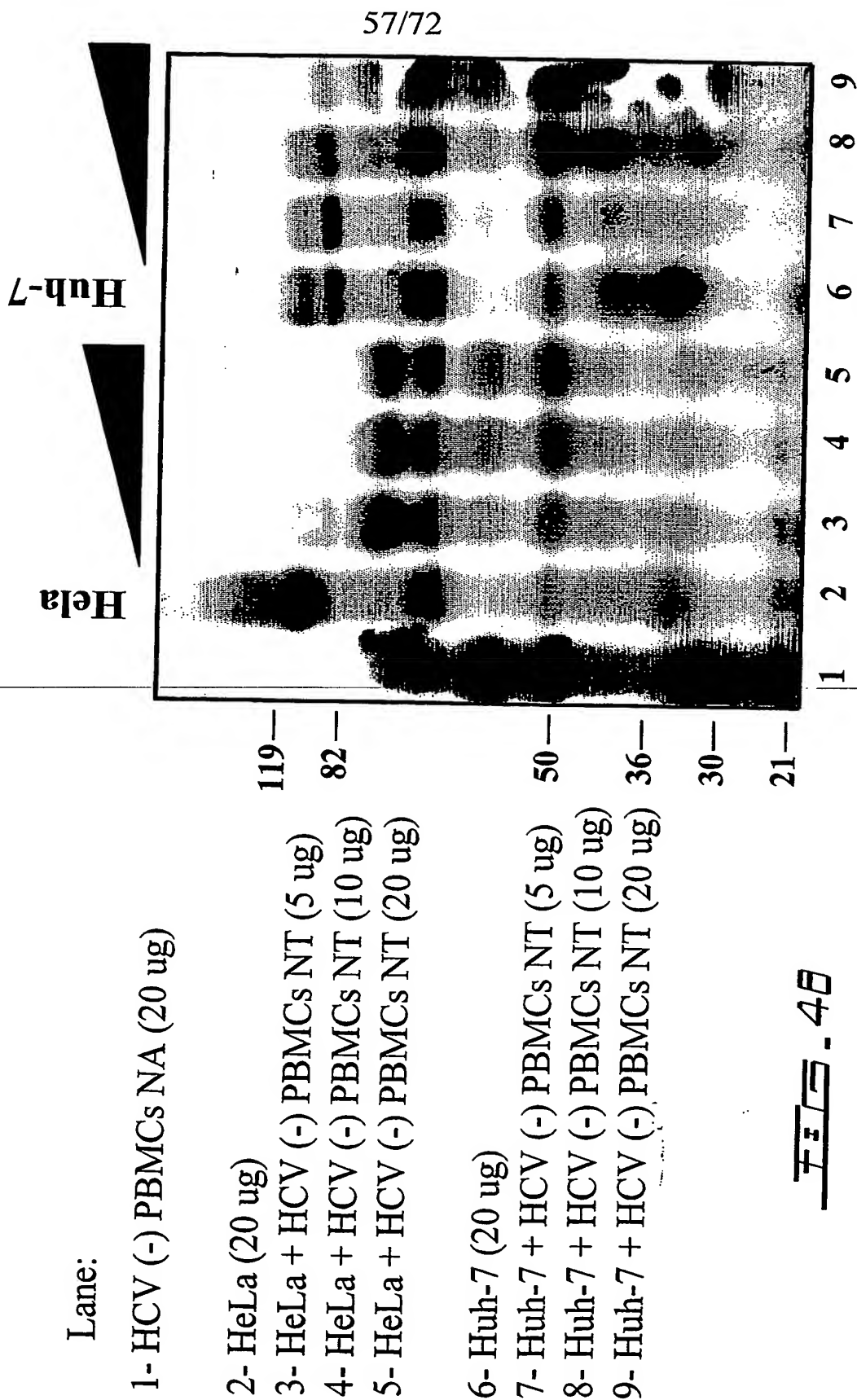
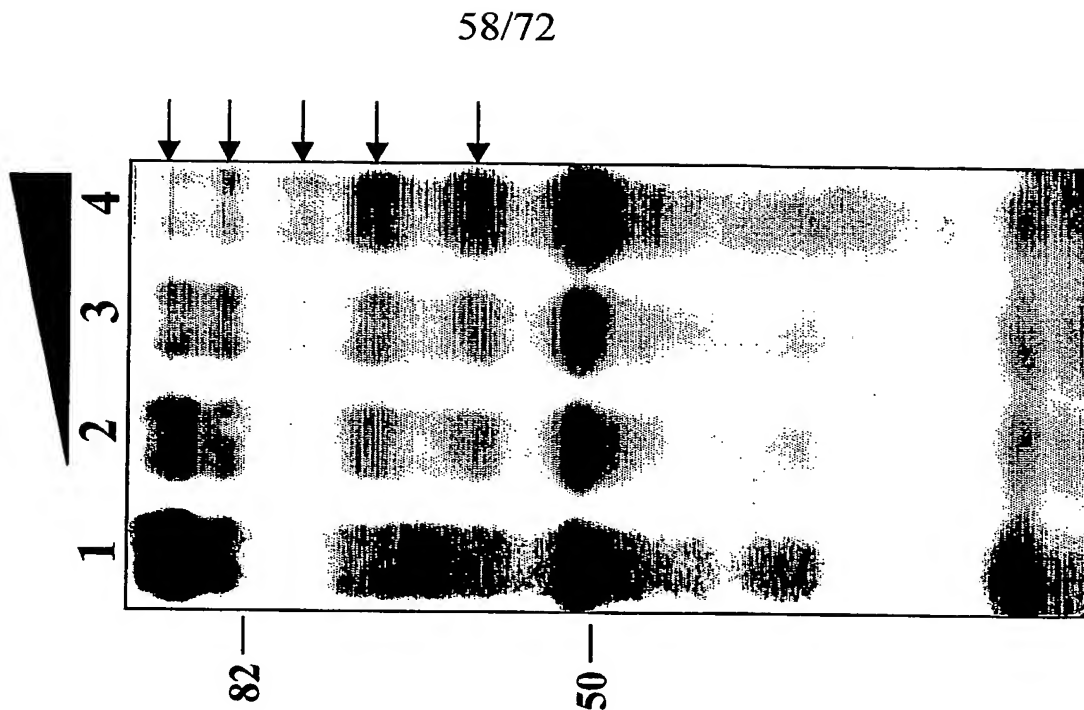


FIG. 4B

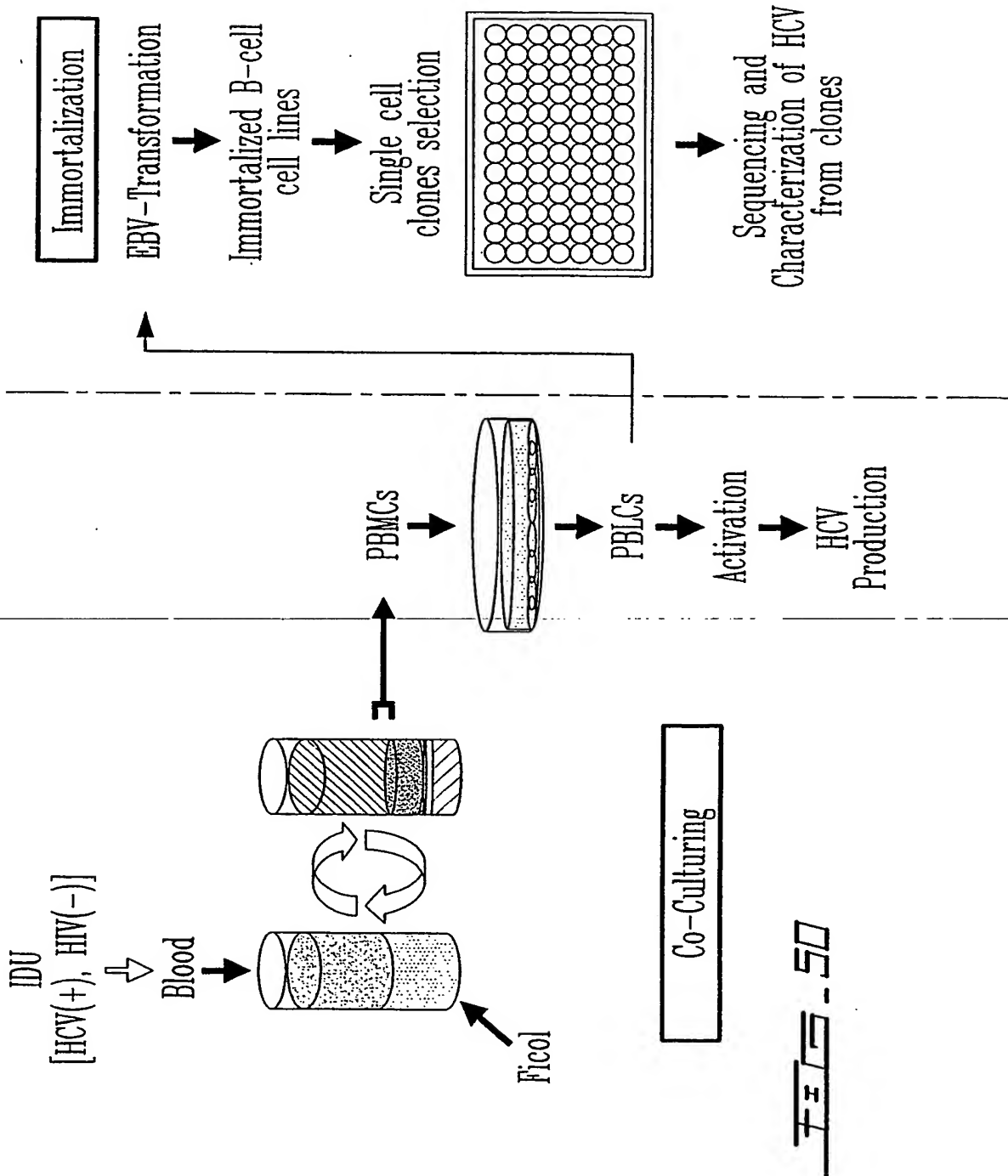


Lane:

- 1- Huh-7 (20ug)
- 2- Huh-7 + HCV (-) PBMCs NT (5ug)
- 3- Huh-7 + HCV (-) PBMCs NT (10ug)
- 4- Huh-7 + HCV (-) PBMCs NT (20ug)

Fig. 49

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# HCV(+)-EBV-Transformed B-Cells.

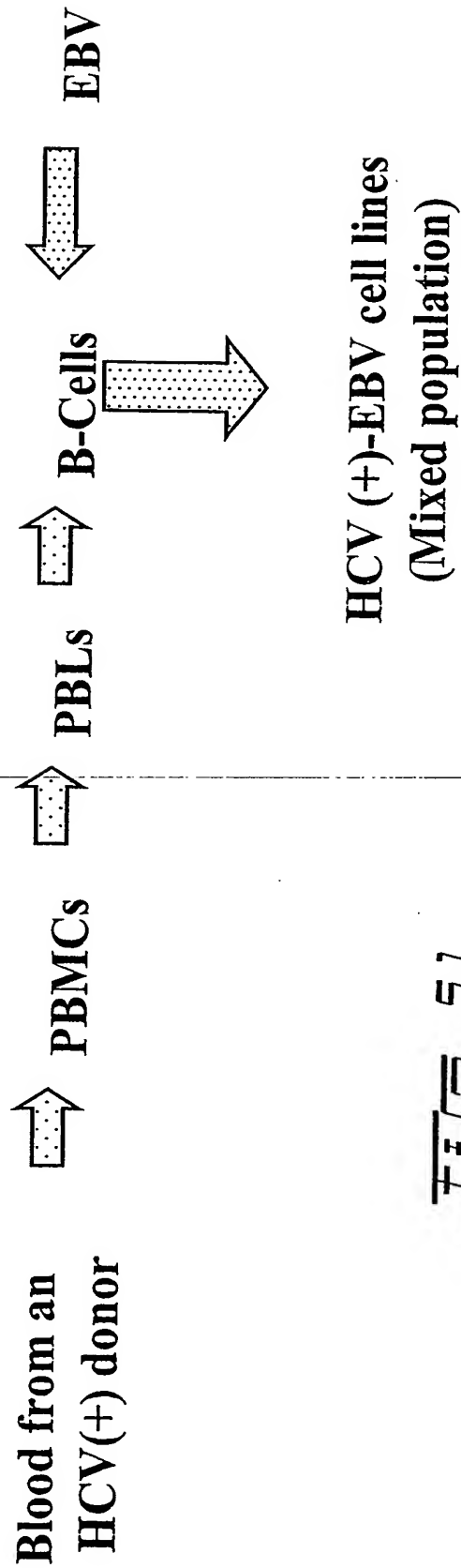


FIG. 51

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HCV RNA is detected in mixed population of EBV-transformed B-cells

### HCV (+) Strand RNA

Cell line	Non-Stimulated cells RNA Copies /10 <sup>6</sup> cells	Stimulated cells RNA Copies /10 <sup>6</sup> cells
EBV-1	4.66x10 <sup>5</sup>	2.33x10 <sup>6</sup>
EBV-2	2.77x10 <sup>5</sup>	7.91x10 <sup>4</sup>
EBV-3	3.96x10 <sup>6</sup>	4.02x10 <sup>5</sup>
EBV-4	2.03x10 <sup>6</sup>	1.57x10 <sup>6</sup>
EBV-6	1.41x10 <sup>6</sup>	4.32x10 <sup>5</sup>
EBV-HCV (-)	0	0

### GAPDH mRNA

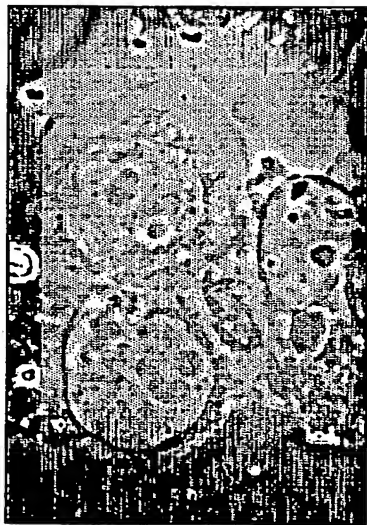
Cell line	Non-Stimulated cells RNA Copies /10 <sup>6</sup> cells	Stimulated cells RNA Copies /10 <sup>6</sup> cells
EBV-1	2.23x10 <sup>8</sup>	2.19x10 <sup>8</sup>
EBV-2	8.73x10 <sup>8</sup>	2.25x10 <sup>8</sup>
EBV-3	1.83x10 <sup>9</sup>	1.77x10 <sup>9</sup>
EBV-4	5.48x10 <sup>8</sup>	3.79x10 <sup>8</sup>
EBV-6	1.26x10 <sup>9</sup>	9.42x10 <sup>8</sup>
EBV-HCV (-)	9.27x10 <sup>7</sup>	3.62x10 <sup>8</sup>

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Control EBV-HCV (-); anti-Core

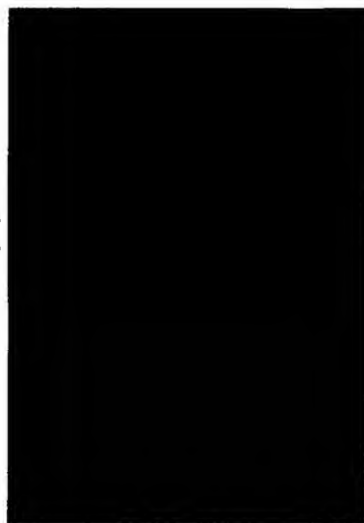
Phase



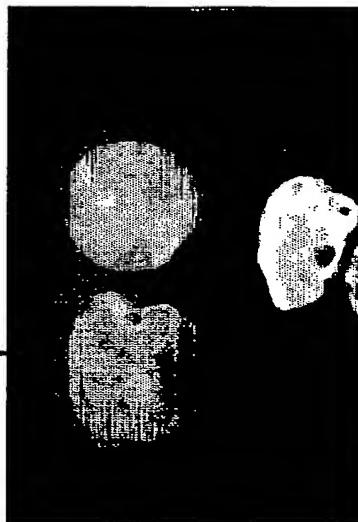
Dapi



Anti-Core



Dapi/Anti-Core



Phase/Dapi/Anti-Core



FIG. 53A

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Detection of Core in EBV-2

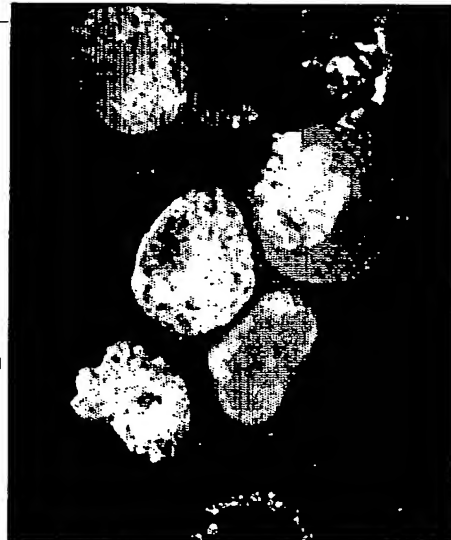
Phase



Anti-Core



Dapi/Anti-Core



Phase/Dapi/Anti-Core



FIG. 53B

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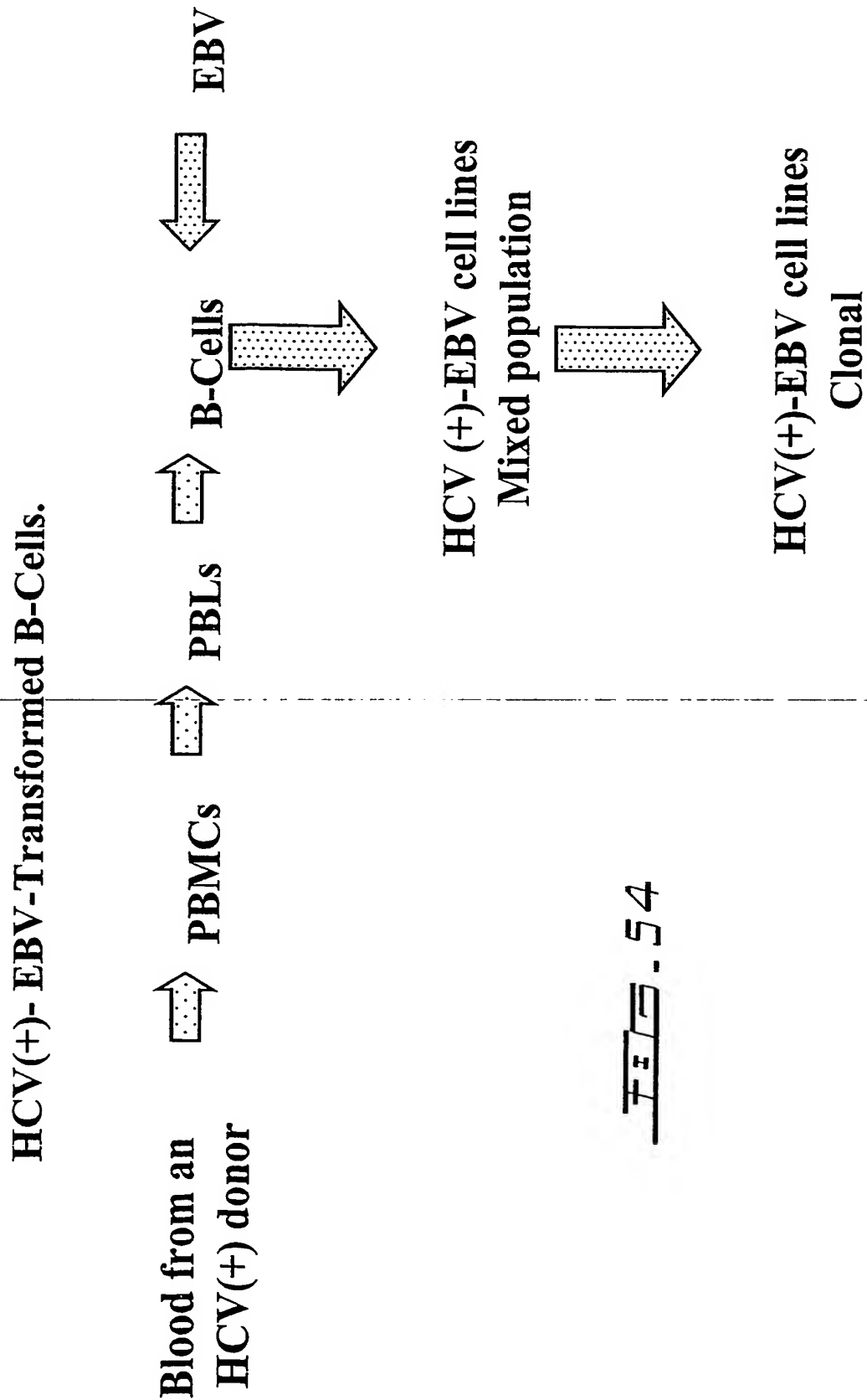
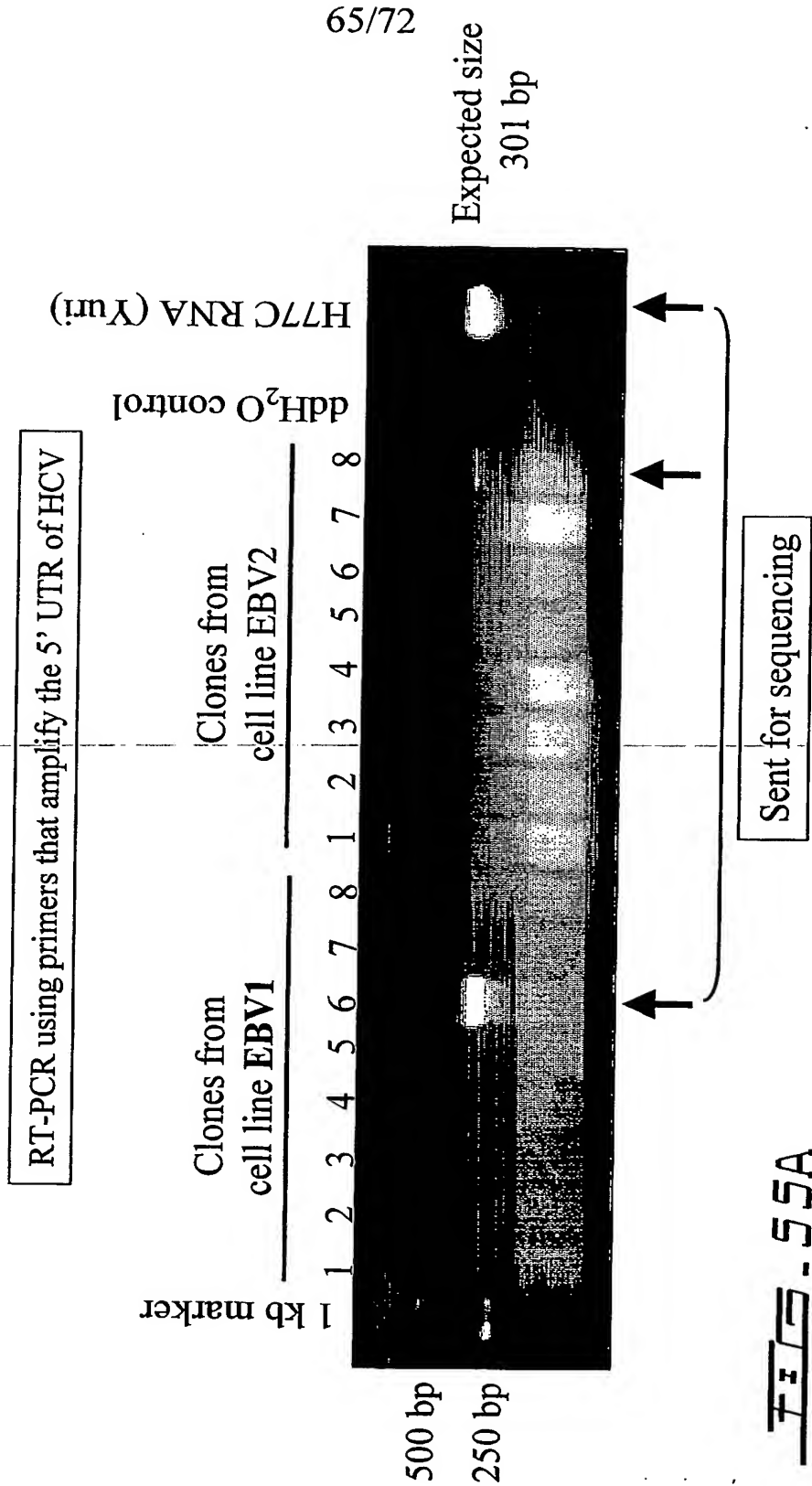


FIG. 54





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Alignment: H77C (RT-PCR positive control) sequence (top)/  
EBV1 clone 6 sequence (bottom)

CACTCCCCTGTGAGGA	ACTACTGTCTTCACGCAGAAAGCGTCTAGCCATGGCGT	
CACTCCCCTGTGAGGA	ACTACTGTCTTCACGCAGAAAGCGTCTAGCCATGGCGT	
TAGTATGAGTGTGTCG	TCCAGCACCCCTCCCGGAGAGCCATAGTGTC	
TAGTATGAGTGTGTCG	TCCAGCACCCCTCCCGGAGAGCCATAGTGTC	
TGCGGAACCGGTGAGT	ACACCGGAATTGCCAGGACCGGGTCCTTTCTTGGATAA	<u>G</u>
TGCGGAACCGGTGAGT	ACACCGGAATTGCCAGGACCGGGTCCTTTCTTGGATTAA	
ACCCGCTCA	CATGCCCTGGAGATTGGGCGTGCCCCCGCAAGACTGCTAGCCGAGTAG	
ACCCGCTCA	-ATGCCCTGGAGATTGGGCGTGCCCCCGCGAGACTGCTAGCCGAGTAG	
TGTTGGGTCGCGAAAGG	CCTGTGTGTA	
TGTTGGGTCGCGAAAGG	CCTGTGTGTA	

Blue: sequence from virus in the serum (MLL-005).

~~77C-55B~~

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Alignment: H77C (RT-PCR positive control) sequence (top)/  
EBV2 clone 8 sequence (bottom).

CCAGGACCCCCCTCCCGGAGAGCCATAGTGGTCTGCGGAACC  
CCAGGACCCCCCTCCCGGAGAGCCATAGTGGTCTGCGGAACC

GGTGAGTACACCGGAATTGCCAGGACGACCGGGTCCTTTCTTGG  
GGTGAGTACACCGGAATTGCCAGGACGACCGGGTCCTTTCTTGG

ATAAACCCGCTCAATGCCCTGGAGATTGGGCGTGCCCCCGCAAG  
ATAAATCCGCTCAATGCCCTGGAGATTGGGCGTGCCCCCGCAAG

ACTGCTAGCCGAGTAGTGTGGGTCGCGAAAGGCCTTGTTGGTAC  
ACTGCTAGCCGAGTAGTGTGGGTCGCGAAAGGCCTTGTTGGTAC

TGCCTGATAGGTGCTTGCGAGTGCCCCCGGAGGTCTCGTAGAC  
TGCCTGATAGGTGCTTGCGAGTGCTCCGGGAGGTCTCGTAGAC

CGTGCA  
CGTGCA

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H77C RNA (Yunt)

ddH<sub>2</sub>O control

EBV1  
Clone 7  
(1.7)

EBV1  
Clone 6  
(1.6)

H77C RNA (Yunt)

EBV1  
Clone 5  
(1.5)

EBV1  
Clone 4  
(1.4)

EBV1  
Clone 3  
(1.3\*)

- A B C D - A B C D

- A B C D - A B C D

- A B C D - A B C D

- A B C D - A B C D



ddH<sub>2</sub>O control  
H77C RNA

EBV9  
Clone 4  
(9.4\*)

EBV9  
Clone 2  
(9.2\*)

EBV2  
Clone 4  
(2.4\*)

EBV2  
Clone 2  
(2.2\*)

H77C RNA

- A B C D - A B C D

- A B C D - A B C D

- A B C D - A B C D

- A B C D - A B C D



sequencing

- = clone alone (not diluted with other cells)  
A= diluted 1:10 with MT4 cell line (HTLV1 transformed T cells)  
B= diluted 1:10 with BJAB cell line (ATCC non-EBV transformed B cells)  
C= diluted 1:10 with HLA 006 cell line (EBV transformed HCV- PBLs)  
D= diluted 1:10 with JAM cell line (EBV transformed HCV- PBLs)

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Alignment of all 9.2 sequences

H77C	CACTCCCCTGTGAGGAACTACTGTCTTCACGCAGAAAGCGTCT
9.2 final seq	CACTCCCCTGTGAGGAACTACTGTCTTCACGCAGAAAGCGTCT
9.2a final seq	CACTCCCCTGTGAGGAACTACTGTCTTCACGCAGAAAGCGTCT
9.2b final seq	CACTCCCCTGTGAGGAACTACTGTCTTCACGCAGAAAGCGTCT
9.2c final seq	CACTCCCCTGTGAGGAACTACTGTCTTCACGCAGAAAGCGTCT
9.2d final seq	CACTCCCCTGTGAGGAACTACTGTCTTCACGCAGAAAGCGTCT
H77C	AGCCATGGCGTTAGTATGAGTGTCGTGCAGCCTCCAGGACCCCC
9.2 final seq	AGCCATGGCGTTAGTATGAGTGTCGTGCAGCCTCCAGGACCCCC
9.2a final seq	AGCCATGGCGTTAGTATGAGTGTCGTGCAGCCTCCAGGACCCCC
9.2b final seq	AGCCATGGCGTTAGTATGAGTGTCGTGCAGCCTCCAGGACCCCC
9.2c final seq	AGCCATGGCGTTAGTATGAGTGTCGTGCAGCCTCCAGGACCCCC
9.2d final seq	AGCCATGGCGTTAGTATGAGTGTCGTGCAGCCTCCAGGACCCCC
H77C	CCTCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACAC
9.2 final seq	CCTCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACAC
9.2a final seq	CCTCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACAC
9.2b final seq	CCTCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACAC
9.2c final seq	CCTCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACAC
9.2d final seq	CCTCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACAC

- = clone alone (not diluted with other cells)

a= diluted 1:10 with MT4 cell line (HTLV1 transformed T cells)

b= diluted 1:10 with BJAB cell line (ATCC non-EBV transformed B cells)

c= diluted 1:10 with HLA 006 cell line (EBV transformed HCV- PBLs)

d= diluted 1:10 with JAM cell line (EBV transformed HCV- PBLs)

Red= Variation with respect to clone 9.2

~~FIG~~ - 57A

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## Alignment of all 9.2 sequences

H77C	CGGAATTGCCAGGACGACCGGGTCCTTTCTTGGATAAACCCGCT
9.2 final seq	CGGAATTGCCAGGACGACCGGGTCCTTTCTTGGATAAACCCGCT
9.2a final seq	CGGAATTGCCAGGACGACCGGGTCCTTTCTTGGAT <u>TA</u> AACCCGCT
9.2b final seq	CGGAATTGCC <u>GGG</u> A <u>GAC</u> TGGGTCCTTTCTTGGATAAACCC <u>A</u> CT
9.2c final seq	CGGAATTGCCAGGACGACCGGGTCCTTTCTTGGATAAACCCGCT
9.2d final seq	CGGAATTGCCAGGACGACCGGGTCCTTTCTTGGAT <u>TA</u> AATCCGCT
H77C	CAATGCCTGGAGATTGGGCGTGCCCCCGCAAGACTGCTAGCCG
9.2 final seq	CAATGCCTGGAGATTGGGCGTGCCCCCGCAAGACTGCTAGCCG
9.2a final seq	CAATGCCTGGAGATTGGGCGTGCCCCCGC <u>G</u> AGACTGCTAGCCG
9.2b final seq	C <u>TA</u> TGCCCGG <u>CC</u> ATTGGGCGTGCCCCCGCAAGACTGCTAGCCG
9.2c final seq	CAATGCCTGGAGATTGGGCGTGCCCCCGCAAGACTGCTAGCCG
9.2d final seq	CAATGCCTGGAGATTGGGCGTGCCCCCGC <u>G</u> AGACTGCTAGCCG

FIG. 57B

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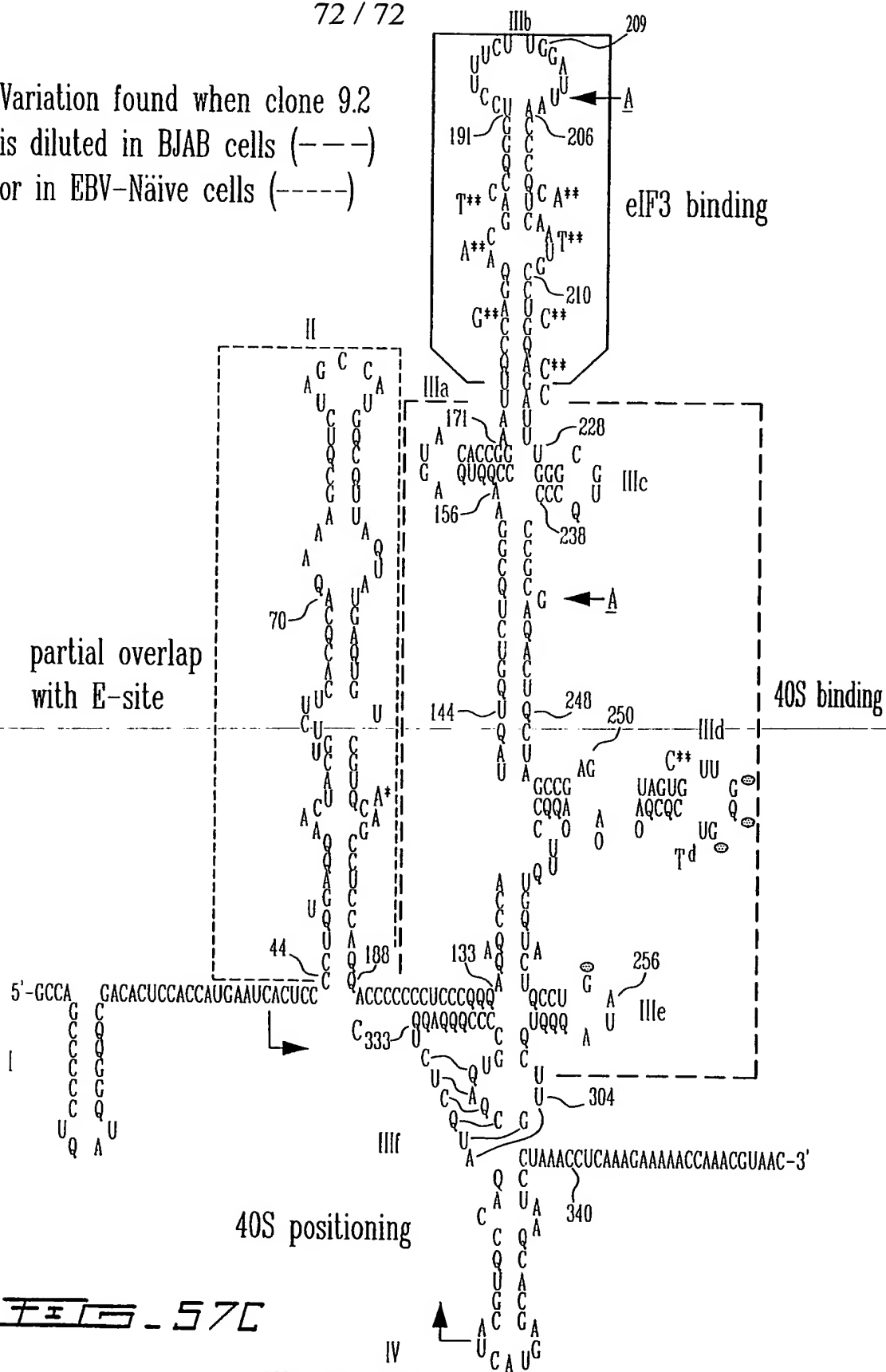
## Alignment of all 9.2 sequences

H77C	AGTAGTGTGGTCCGGAAGGCCCTTGTGGTACTGCCTGATAGG
9.2 final seq	AGTAGTGTGGTCCGGAAGGCCCTTGTGGTACTGCCTGATAGG
9.2a final seq	AGTAGTGTGGTCCGGAAGGCCCTTGTGGTACTGCCTGATAGG
<b>9.2b final seq</b>	AGTAGC <u>CG</u> TGGGT <u>T</u> GC GAAAGGCCCTTGTGGTACTGCCTGATAGG
9.2c final seq	AGTAGTGTGGTCCGGAAGGCCCTTGTGGTACTGCCTGATAGG
9.2d final seq	AGTAGTGTGGTCCGGAAGGCCCTTGTGGTACTGCCTGATAGG
H77C	GTGCTTGCGAGTGCCCCGGGAGGTCTCTGTAGACCGTGCA
9.2 final seq	GTGCTTGCGAGTGCCCCGGGAGGTCTCTGTAGACCGTGCA
9.2a final seq	GTGCTTGCGAGTGCCCCGGGAGGTCTCTGTAGACCGTGCA
<b>9.2b final seq</b>	GTGCTTGCGAGTGCCCCGGGAGGTCTCTGTAGACCGTGCA
9.2c final seq	GTGCTTGCGAGTGCCCCGGGAGGTCTCTGTAGACCGTGCA
9.2d final seq	GTGCTTGCGAGTGCCCCGGGAGGTCTCTGTAGACCGTGCA

FIG. 57B (Cont.)

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Variation found when clone 9.2  
is diluted in BJAB cells (---)  
or in EBV-Näive cells (-----)



**FIG. 57C**

➔ = Primers

IRES structure from Sarnow P (2003), J Virol. 77, 2801-6

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